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CARCINOMA OF THE TUBES AND OVARIES SECONDARY TO CARCINOMA OF THE BODY OF THE UTERUS *

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It is the purpose of this paper to present the results of the study of a small series of judged primary carcinoma of the body of the uterus associated with judged secondary carcinoma, either in the tube, the ovary, or in both organs. An attempt was made to determine the pathogenesis of the secondary carcinomas, with special reference to the justification of the theory that cancer cells escaping from the uterine tumor may be transported through the lumen of the tube and may become implanted in the tubal mucosa and on the surface of the ovaries and peritoneum.

It is a reasonable assumption that carcinoma in any two or all three of these organs may arise in various ways. The close anatomical relations between the uterus, tubes and ovaries, often made more intimate by an advanced carcinoma in one, may readily permit the continuous extension of the growth from one organ to another through normal preëxisting structures, or by the affected organ becoming adherent to one of the others. The common lymphatic and venous circulation of the three organs offers channels through which carcinoma may extend from one organ to another by continuous permeation; or emboli of cancer cells escaping into the main vessels may be diverted to the tubes or ovaries in channels where the stream is often sluggish, especially if there is the least temporary or permanent obstruction beyond. The lumina of the tubes afford avenues not only for the transportation of sperm and ova, but also for bacteria and cancer cells.

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The common ancestral origin of the germinal epithelium of the ovary, from which many of the ovarian carcinomas directly or indirectly arise, and that of the tubal and uterine epithelium offers a foundation for the simultaneous development of carcinoma in any two or all three of these organs, namely, carcinoma of multicentric origin in closely allied organs. Since primary carcinoma occurs in all three organs it is natural to assume that, in some instances of this disease in two or even in all three, the growths in the different situations might be independent of each other. Nevertheless, carcinomas of multicentric origin in the same or different organs are of infrequent occurrence. When multiple carcinomas of the same histological structure are present in an individual, it usually indicates a primary growth in one situation with secondary tumors in the others.

It is generally accepted by both clinicians and pathologists that carcinoma usually spreads by continuous extension and by metastases through the lymphatics. Therefore it is quite natural to attempt to explain the presence of this growth in any two or all three of these organs as having arisen from a primary neoplasm in one with the development of secondary tumors in the other situations, as a result of continuous extension of the primary tumor or lymphatic metastasis.

Observation of conditions found at operation, together with a careful study of the specimen removed, should enable one, definitely, to determine whether or not the tumors in the different organs are continuous. On the other hand, the evidence of the pathogenesis of any seemingly metastatic growth is, at best, merely circumstantial.

When the growth in the different organs is in contact with the epithelium of these organs the multicentric theory is alluring if continuous extension can be excluded and evidence of metastasis through well recognized channels is not present.

The Status of the Transtubal Implantation of Carcinoma

Reichel,[1] in the year 1888, reported two cases of ovarian carcinoma, each associated with a small carcinoma of the uterine mucosa. In the discussion of the etiology of the uterine carcinomas in these two cases he introduced the theory that they arose from the implantation of cancer cells, which had escaped from the ovarian carcinoma

through the lumina of the tubes, on the surface of the uterine mucosa. He compared their pathogenesis with the phenomenon of the passage of the fertilized ovum through the tube and its implantation in the uterine mucosa.

Sitzenfrey,[2] in the year 1908, reported a case of carcinoma of the uterus in which free particles of the uterine growth were found in the lumen of one of the tubes without the presence of carcinoma of the tubal mucosa. He believed that secondary tubal carcinoma may arise from this source and that with a further migration of these particles implantations of this growth on the surfaces of the ovary and peritoneum may arise.

In 1922 Schiller [3] reported a similar case. He agreed with Sitzenfrey as to the possible etiology of secondary tubal, ovarian and peritoneal carcinoma from this source.

In 1924 I published a paper [4] entitled: "Benign and Malignant Endometrial Implants in the Peritoneal Cavity and their Relation to Certain Ovarian Tumors." It was shown, in that paper, that fragments of malignant endometrial tumors, at times, escaped into the lumina of the tubes. Evidence was presented indicating that implantations of these particles sometimes occurred in tubal mucosa, and on the surfaces of the ovaries and peritoneum. Two cases reported in the previous paper will again be presented in the present one, with additional photomicrographs showing the secondary tubal and ovarian tumors. Later in the same year Norris and Vogt [5] reported an instance of an early metastasis of carcinoma on the surface of the ovary from carcinoma of the uterus. They believed the ovarian carcinoma arose from the implantation of cancer cells escaping through the lumen of the tube during a diagnostic curettage six weeks before.

In 1927 Novak,[6] in a paper entitled "Ovarian Metastasis with Cancer of the Uterine Body: Is Transtubal Implantation an Important Factor?" presents an excellent summary of the evidence that the lymphatics are the chief channels for metastases of uterine carcinoma to the ovary and *vice versa*. He most emphatically endorses these channels as avenues for the dissemination of carcinoma from the uterus to the ovary, and just as emphatically denounces the transtubal route. He states: "The cases reported by Sampson of supposed implantation cancer of the ovary are far more logically explained as due to lymphatic dissemination."

In 1930 Robinson,[7] in an interesting paper on primary and secondary ovarian carcinoma, denounces the implantation theory as applied to the origin of metastatic ovarian carcinoma secondary to the intestinal tract, and warmly supports the lymphatic route. He states: "The soundest of all theories formulated about the manner and method of cancer metastasization is the one of the lymph and vessel routes, and this theory is easily and frequently verified. In fact, some authorities admit of no other possibility." Robinson believes that the simultaneous appearance of malignant papillary tumors, within ovarian cysts and upon the surface of the ovaries, and similar growths of the uterine and tubal mucosa, as well as the serosa covering these organs, is due to the same biological factors influencing variously located and metamorphosed epithelial centers of a common genetic source.

In 1932 Offutt [8] published the results of an analysis of a series of cases from the Mayo Clinic in which carcinoma was present in both the ovaries and uterus. There were 520 cases of carcinoma of the body of the uterus and 616 of papillary cystadenocarcinoma of the ovaries, in which operation had been performed in the clinic from 1913 to 1930. In fifty-three of these cases carcinoma was present in both organs. In five of these cases the uterus was judged to be the site of the primary neoplasm. Carcinoma was present in a Fallopian tube in three of these. In sixteen, one or both ovaries were judged to be the site of the primary growth, in eight of which one or both tubes also contained carcinoma. In thirty-one cases the situation of the primary growth could not be determined, in four of these carcinoma was present in one tube. Offutt discusses the various means by which this growth may extend from the uterus to the ovary and *vice versa*. She emphasizes the importance of the transtubal route in the group of cases reported by her. In several instances cancer cells were found in the lumen of the tube of which the blood vessels and lymph channels were apparently normal.

This brief reference to some of the literature dealing with the transtubal migration of cancer cells and the origin of secondary carcinoma of the uterus, tubes and ovaries from this source must impress one, not only with the difficulty of properly evaluating this channel, but also the natural difference of opinion that must arise as to its importance, and as to whether metastases ever occur in this way.

I heartily agree with the majority of pathologists who believe that carcinoma is usually disseminated from its primary focus through the lymphatics. However, there is one striking exception to this rule and that occurs in certain types of ovarian carcinoma where implantations on the peritoneum arise from the growth of cells escaping from the primary tumor into the peritoneal cavity. Even in these cases the lymphatics also may be invaded, not only by the primary tumor, but also by secondary tumors on the peritoneum which may have arisen from the primary one by implantation. We must not lose sight of the fact that a secondary tumor may possess the same or even greater potentialities of invasion and dissemination than the growth from which it arose by direct extension or metastasis.

It is generally accepted by both clinicians and pathologists that, as compared with carcinoma of the uterine cervix, metastases to the retroperitoneal lymph nodes rarely occur in carcinoma of the body of the uterus.

It is not my purpose to belittle the importance of the lymphatics or veins as avenues for the dissemination of carcinoma of the body of the uterus, or to minimize the incidence of carcinoma of multicentric origin, or over-emphasize the part played by the lumina of the tubes in conveying cancer cells from the uterine tumor to that organ and thence to the surface of the ovary and peritoneum. However, I desire to present what was found in this series of cases, to give my own reactions and gladly leave others to draw their conclusions, not only from my observations but, of greater importance, from the study of material from other cases similar to those which I am to report.

The closest available analogy we have to the implantation of cancer cells of uterine origin on the surface of the ovary and peritoneum is the implantation of cancer cells of ovarian origin on the peritoneum, since genetically the peritoneal mesothelium, the surface epithelium of the ovary and the uterine epithelium have a common ancestor. The results of a previous study [9] of implantation peritoneal carcinomatosis of ovarian origin demonstrated that it arises from the repair of injuries to the peritoneum caused by cancer cells that have escaped into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation. The various stages in this repair, as well as the laws governing the same, are similar to those encountered in the repair of tissues injured by foreign bodies and in the taking of

skin grafts — namely, the healing of wounds. The histological structure of these implants varies with the reaction of the peritoneal tissues before and after the fixation of the cancer cells and with the activity of the latter. As a result carcinoma becomes embedded in the peritoneal scar, encapsulated on its surface, enmeshed in adhesions, or like a surgical skin graft grows on the surface of the peritoneum without encapsulation.

The principles governing the implantation of uterine carcinoma in the mucosa of the tubes should be the same as those governing the taking of a small skin graft, bearing in mind that the cancer graft is derived from epithelium having the same ancestor as the tubal epithelium. The cancer cells must be viable, there must be a break in the epithelial lining of the tube already present or possibly created by the carcinoma, and the graft must be held in place or at least not disturbed until it becomes firmly attached. It is conceivable that such conditions would be encountered in the tube more often than in the intestines or urinary tract, including the bladder. It is also conceivable that cancer cells of uterine origin once grafted in tubal mucosa might bear the same relation to the normal tubal epithelium about it as carcinoma primarily arising from that epithelium. If true, it would be impossible to determine from its structure whether a given tubal carcinoma arose from grafted cancer cells or from a differentiation of tubal epithelium.

Material and Methods of Study

From 1921 to 1932 inclusive, 183 patients with carcinoma of the body of the uterus have been operated upon in the gynecological service of the Albany Hospital. The material from nineteen of these cases was chosen for the present paper and also from one additional case operated upon in 1933. Thirteen of the twenty cases included all instances of carcinoma of the body of the uterus with a similar growth in the tube, ovary or both organs, in which the growth in the uterus was judged to be the primary one.

The criteria upon which this opinion was formed was not only the size and apparent age of the uterine tumor and its histological structure (corresponding with that of uterine carcinoma without carcinoma in the tubes and ovaries), but also the relatively smaller size and apparently younger age of the associated tumors in the other organs. In addition, evidence must be present indicating that carcinoma actually has or may have reached these organs from the uterine growth. I realize that any or even all of these criteria may not be sufficient in a given case for a positive diagnosis of the site of the primary neoplasm. A secondary tumor may be larger and more invasive than its parent and therefore apparently older; and primary carcinoma of the tube and ovary may histologically be

indistinguishable from carcinoma arising in the endometrium. Also, the evidence of the pathogenesis of a metastatic tumor, no matter how convincing, is at best circumstantial and even may be misleading.

The cases of obvious primary carcinoma of the tubes and of large or extensive ovarian carcinomas were not included, even though there was associated with the latter an extensive carcinoma of the uterus. In some of the latter group the primary growth might well have been in the uterus. The relation between carcinomas in these organs is much more difficult to determine when the tumors in both situations are either of the same size or advanced.

The findings in the remaining seven cases of carcinoma of the body of the uterus were chosen on account of their bearing on the pathogenesis of the conditions present in the other cases.

The study of this problem begins in the operating room. On exposing the pelvic contents a careful examination is made to determine whether or not metastases are present. The ovaries and tubes, and especially the fimbriae of the latter, are inspected for any abnormalities suggesting carcinoma in these structures. If present the tubes and ovaries are first removed and immediately placed in formalin. This enables one to obtain material which otherwise might be traumatized during or after the operative removal of the uterus and likewise minimizes the danger of disseminating cancer cells from them into the operative field When the tubes and ovaries appear normal each tube is ligated just back of the fimbriae to prevent any carcinoma possibly present in the lumen of the tube, or later forced into it by operative manipulation, from escaping through the distal end of the tube. The ovarian and uterine vessels are either doubly ligated and severed between the ligatures, or else ligated and clamped proximal to the ligature before cutting them. This is done to prevent any cancer emboli possibly present in the lumina of the lymphatics and veins from escaping into the field of operation. After freeing the cervix and upper portion of the vagina a right-angle clamp is placed across the vagina below the cervix and a vaginal douche is given before severing the vagina to prevent further soiling of the field of operation.

All specimens are examined before they leave the operating room. Should they present unusual features sketches are made and these are carefully labelled. Specimens not presenting unusual features are sent, as such, to the general pathological laboratory, where they are described and tissue is removed from the uterus, tubes and ovaries, fixed in Zenker's fluid, embedded in paraffin, and cut and stained as a routine procedure.

The routine examination of pathological material is of the greatest value for records and diagnostic purposes, but often is not sufficient for the intensive study of any special problem. Through the courtesy of Dr. Victor C. Jacobsen I have been permitted to retain portions of specimens in which I have been especially interested, saving for him sufficient material for diagnosis and laboratory records.

I employ formalin for fixing the tissue and block it myself. The blocks (often large) are embedded in celloidin rather than paraffin, as it causes less shrinkage and fewer artifacts after formalin fixation. One can then study the surface of the block as the sections are cut. My technician is instructed how the blocks are to be mounted and cut, and, most important, what I wish to ascertain in each block. Much may be learned by a well trained technician while watching the surface of the block as the sections are cut. From time to time, while cutting the block, sections are stained to make sure of the conditions present. If the technician is in doubt the block is not cut further until I have had an opportunity to study the stained sections. All sections are saved until the entire block has been cut and

sections studied. In many situations serial sections are made. This has proved to be of the greatest importance in many instances. Often I have wished for serial sections after the block has been cut. Since two sections cannot be examined under the microscope by one person at the same time, photomicrographs are used extensively by me in the study of these problems. I find them of great assistance in the comparative study of the findings in different sections.

In each instance the methods employed in the study of this material were initiated by the interest aroused by conditions observed at operation or in the inspection of the fresh specimen after its removal. This led to the intensive study of some portions of the specimen and a relatively casual examination of others. As a result there is a lack of uniformity in the manner and thoroughness of the study of all the specimens, and even of all parts of those more or less intensively studied. For these reasons these studies are of little or no statistical value.

Case Reports

Case 1. Advanced adenocarcinoma of the body of the uterus, which had extended through the posterior uterine wall with resulting invasion of the omentum and the peritoneum of the mesosigmoid which had become adherent over this area; metastases to the subperitoneal tissues of the left uterine cornu with subsequent extension through the overlying peritoneum.

Albany Hospital No. 8029–30. The patient, aged 56 years, complained of uterine bleeding of 7 months duration. A diagnostic curettage was done at the Albany Hospital Nov. 6, 1930. Because of difficulty in persuading the patient to have a major operation, the latter was not done until Nov. 12, 1930. At that operation the omentum and mesosigmoid were found to be adherent to the fundus and posterior surface of the uterus. A small amount of blood-tinged fluid was present in the posterior cul-de-sac. Some of this was removed for microscopic examination of its cellular contents. No gross evidence of metastases was detected. The portion of the omentum adherent to the uterus was excised and the mesosigmoid was freed from the uterus by removing the portion of its peritoneum, which was adherent to the latter. The entire uterus, both tubes, ovaries and appendix were removed. The patient made a satisfactory convalescence but died in a year's time from carcinoma.

A study of the specimen removed (see Figs. 1 to 5 inclusive, with their legends) demonstrated the following: (*a*) the variations in histological type that different portions of the same cancer may present; (*b*) the penetration of the uterine wall by the tumor and subsequent invasion of structures becoming adherent to it over this area, the possibility that carcinoma, which has thus penetrated the uterine wall, may escape into the peritoneal cavity and become implanted on the surface of the peritoneum; (*c*) the presence of subperitoneal metastases of the uterus with cancer cells in the nearby lymph vessels,

the extension of carcinoma in these metastases to the surface of the peritoneum and its possible subsequent escape into the peritoneal cavity; (*d*) the presence of clumps of possible cancer cells in peritoneal fluid which may have escaped into the peritoneal cavity through the sources already described or through the patent tubes during the curettage six days before the major operation. Carcinoma was not found in the tubes and ovaries.

CASE 2. Advanced adenocarcinoma of the body of the uterus with extension through the posterior uterine wall and subsequent adhesions between it and the sigmoid and terminal loop of the ileum; carcinoma of the left ovary from continuous extension of the uterine growth; subperitoneal metastases of the posterior uterine wall and left tube, and peritoneal carcinomatosis limited to the posterior cul-de-sac.

Albany Hospital No. 8781–31. The patient, aged 72 years, complained of pain and uterine bleeding of 3 months duration. A preliminary diagnostic curettage was not done. At operation Nov. 28, 1931, the uterus was found to be enlarged, due to multiple small myomata, and was slightly adherent to the sigmoid and terminal loop of the ileum. Metastases to the retroperitoneal lymph nodes were not detected. The entire uterus and both tubes and ovaries were removed. In doing so it was noted that peritoneal carcinomatosis was present in the posterior cul-de-sac. The patient made a satisfactory convalescence and felt better for several months after the operation, but died in a little over a year's time.

A study of the specimen removed (see Figs. 6 to 17 inclusive, with their legends) demonstrated: (*a*) the variations in histological type in the same and different portions of the cancer; (*b*) the diffuse extension of the carcinoma through the uterine wall, and especially through that of the left cornu; (*c*) the invasion of the left ovary by direct extension through the left utero-ovarian ligament; (*d*) subperitoneal metastases of the uterus and left tube, and also a submucosal metastasis of the fimbriae of the left tube. The most interesting and important finding was the extension of the carcinoma through the uterine wall to the surface of the peritoneum, with the development of local peritoneal carcinomatosis from the judged implantation of cancer cells thus escaping into the peritoneal cavity (see Figs. 14, 16 and 17). Since implantation carcinomatosis arose near the site of the penetration of the carcinoma into the peritoneal cavity, a similar phenomenon could well account

for the peritoneal carcinomatosis at a little greater distance, namely, in the posterior cul-de-sac. Carcinoma was not found in the right tube and ovary.

CASE 3. Advanced squamous cell carcinoma of the body of the uterus secondary to that of the cervix, with direct extension to the surrounding structures, metastases to the pelvic lymph nodes and secondary carcinoma of the right tube and ovary, apparently due to continuous lymphatic permeation.

Albany Hospital Nos. 6607–30 and 4727–31. The patient, aged 64 years, was first seen by me Sept. 10, 1930. She had had radium treatment for carcinoma of the cervix in a hospital in another city 6 months before. The cervix was replaced by a necrotic ulcerative mass. The uterus was fixed in the pelvis. The disease was so far advanced that the value of further radium treatment seemed questionable. Nevertheless it was employed. At first the palliative relief was quite marked. The patient was readmitted June 16, 1931, for uncontrollable uterine bleeding. An exploratory abdominal incision was made under spinal anesthesia June 30, 1931, with the plan of ligating the blood supply to the uterus and thus stopping the bleeding. The uterus was about three times its normal size and fixed in the pelvis. One could easily follow the white, distended, subperitoneal lymphatics of the right uterine cornu out between the layers of the broad ligament, where they were joined by similarly dilated lymphatics of the right tube and ovary. These lymphatics were evidently filled with carcinoma as with a white injection mass. The retroperitoneal tissues of both sides of the pelvic wall were so infiltrated with the growth that it was impossible to gain access to either the anterior branches of the internal iliac arteries or their uterine branches. The ovarian vessels were enlarged, thus suggesting that the uterus was receiving the greater portion of its blood supply from these sources. The right tube and ovary were removed and the left ovarian vessels and both round ligaments were ligated. The operation did not disturb the patient. The bleeding ceased and never returned. The patient died in June, 1932.

A study of the right tube and ovary (see Figs. 18 and 19, with their legends) demonstrates the location and type of carcinoma of the tube and ovary that may arise from the continuous permeation of the lymphatics by uterine carcinoma.

CASE 4. Advanced adenocarcinoma of the body of the uterus with secondary carcinoma of both tubes, ovaries, round ligaments and peritoneum of the cul-de-sac about the uterosacral ligaments. The secondary growths apparently arose mainly from lymphatic permeation and possibly in some instances from embolic metastases.

Albany Hospital No. 5634–28. The patient, aged 61 years, complained of uterine bleeding of over a year's duration. A preliminary curettage was done, followed by an exploratory incision Sept. 13, 1928. The uterus was enlarged and

fixed in the pelvis, due to extension of the growth through the lymphatics, especially those about the round and uterosacral ligaments with resulting invasion of the peritoneum about the ligaments. Both tubes were enlarged and their walls firm. The ovaries were of normal size. No attempt was made to remove the uterus. The right tube, ovary and left tube were removed and the left ovarian vessels ligated. The patient made a satisfactory convalescence, but died later.

A study of the right tube and ovary (see Figs. 20 to 26 inclusive) demonstrated the permeation of the lymphatics by carcinoma and the origin and type of carcinoma of the tube and ovary secondary to that of the uterus from lymphatic permeation and metastases. We were able to demonstrate, by serial sections, that all the growth in the dilated lymph vessels was not in the form of continuous threads or strands, but that some of it consisted of clumps of cancer cells, namely emboli, floating about in the lumina of the dilated lymphatics. Furthermore, we were able to show that these emboli at times became attached to the lining of these vessels, forming implantations of both the grafted and foreign body (encapsulated) type (Figs. 21 and 23), similar to the metastatic peritoneal tumors frequently found in peritoneal carcinomatosis of ovarian origin.

CASE 5. Advanced adenocarcinoma of the uterus, which had been treated with radium. Two months after the biopsy and radium treatment a papillary metastasis was found at the junction of the vagina and vulva in the scar of a wound of the hymen created at the time of the afore-mentioned operation.

Albany Hospital Nos. 6756–32 and 8462–32. The patient, aged 51 years, complained of pelvic pain radiating down both legs, especially the right one, and uterine bleeding of 4 weeks duration. A biopsy resulting in a diagnosis of carcinoma had been made in a hospital in another city 2 weeks before. The condition was considered to be inoperable at that time. On examination at the Albany Hospital, Sept. 21, 1932, the vaginal canal would just admit one's forefinger (hymen thick and rigid). One could feel that the cervical canal was dilated by a friable growth. On rectal examination the uterus was found to be enlarged and fixed in the pelvis with induration on both sides. Under gas oxygen anesthesia, Sept. 23, 1932, the cervix was exposed and an attempt was made to ascertain the origin of the tumor and its extent. This was only partially successful. It was our impression that while it might have arisen from the mucosa of the cervical canal, it was more likely a carcinoma of the body of the uterus which protruded into the cervical canal. Tissue was obtained for microscopic examination. A small nodule in the vaginal wall below the cervix was excised, thinking it might be a metastasis. It proved not to be one. One hundred milligrams of radium, in one capsule, was introduced through the cervix into the tumor and left in place for 24 hours. The posterior portion of the hymen was found to be torn through into the vagina as a result of the dilatation necessary to expose the cervix. No at-

tempt was made to close this wound but it was packed with gauze to control the bleeding. One hundred milligrams of radium was again inserted in the uterine cavity, Sept. 28, 1932, this time in two capsules of 50 mg. each, tandem formation. The patient was seen again Nov. 28, 1932. She considered herself well; both the pain and bleeding had ceased. A check-up was made in the Albany Hospital Nov. 29, 1932. On exposing the vaginal orifice a small red papilloma was noted at the junction of the posterior vaginal wall with the vulva — the site of the wound of the hymen caused 2 months before. This was excised, believing that while it might be malignant, it was more likely a tuft of granulation tissue which often appears in vaginal wounds that have not completely healed. A curettage was done and carcinoma was not found in the uterus. The uterus seemed smaller and less fixed.

The microscopic examination of the papilloma removed (see Fig. 36, with its legend) demonstrated that it was a papillary adenocarcinoma of the same histological structure as the primary uterine growth. The evidence presented, as well as its histological structure, indicates that it was a metastatic carcinoma of the vagina secondary to that of the uterus, probably caused by the transplantation of cancer cells in the wound of the vagina created two months before.

CASE 6. Advanced adenocarcinoma of the body of the uterus with metastases to the distal end of the left tube, the mucosa of the cervix and the anterior vaginal wall. This case was reported in a previous paper [4] (see Case 1 of that paper).

Albany Hospital No. 89234. The patient, aged 62 years, complained of uterine bleeding which had been more or less constant for 12 years. She had been curetted a year before I saw her. A diagnosis of malignancy was made at that time and the condition was considered inoperable. Since then she had had repeated X-ray treatments. At operation, March 16, 1923, the uterus was found to be enlarged, due to multiple small myomata and an extensive carcinoma. There was no gross evidence of metastases found at operation, other than a thickening of the fimbriated end of the left tube and a nodule in the anterior vaginal wall beneath the urethra. The entire uterus and both tubes and ovaries were removed. The metastasis in the anterior vaginal wall was excised and the vagina treated with radium. The patient made an uninterrupted recovery, has been seen by me several times since then, the last time in June, 1933. At no time have I been able to detect any evidence of a recurrence. Ten years have elapsed since her operation.

A study of the specimen removed (see Figs. 38 to 42 inclusive of present paper, with their legends, as well as the previous report of this case) demonstrated: (*a*) an advanced carcinoma of the body of the uterus, which in several places had extended almost entirely through the wall of the uterus to its serosa; (*b*) the presence of judged

emboli of cancer cells in veins in the peripheral zone of the uterus and in a vein in the tubal wall; (*c*) a carcinoma of the distal end of the left tube, which was judged to be of implantation origin; (*d*) clumps of cancer cells lying free in the lumen of the left tube and the judged implantation of one of these clumps in the tubal mucosa; (*e*) a metastatic carcinoma of the cervical mucosa judged to be of implantation origin; (*f*) a metastasis in the anterior vaginal wall which, from its histological structure, could have been primarily a surface implant, or a lymphatic, or venous metastasis. Carcinoma was not found in the ovaries or in the mucosa of the other tube. The finding of judged cancer cells in the veins of the peripheral zone of the uterine wall and in a vein of the tubal wall might be considered as evidence favoring the origin of the above described metastases through these channels. These may have been of more recent origin, or even caused by the trauma of the operation. The vaginal metastasis is the only one suggesting a possible origin from this source.

CASE 7. Adenocarcinoma of the body of the uterus with multiple carcinomas of the mucosa of the right tube, of various sizes, thus suggesting different ages.

Albany Hospital No. 890–29. The patient, aged 53 years, complained of uterine bleeding of 7 months duration. At operation, Feb. 9, 1929, which had been preceded by a diagnostic curettage, the uterus was found to be slightly enlarged. There was no gross evidence of metastases. The left tube and ovary appeared normal. The right tube was dilated and its fimbriated end occluded and adherent to the right ovary. The entire uterus and both tubes and ovaries were removed. The patient made a satisfactory convalescence and has remained well; was last seen in June, 1933.

Carcinoma was not found in the left tube and ovary. The data, supporting the theory that the multiple carcinomas of the mucosa of the right tube primarily arose from the implantation of cancer cells escaping from the uterine growth through the uterine ostium of the tube, are presented in Figs. 44 to 47 inclusive.

CASE 8. Advanced papillary adenocarcinoma of the body of the uterus, with extension through the wall of the uterus near the level of the internal os almost to its serosa. Carcinoma of the fimbriae of the left tube.

Albany Hospital Nos. 7001–30 and 229–33. The patient, aged 55 years, complained of uterine bleeding of 3 years duration. At operation, Sept. 27, 1930, the uterus was found to be about four times its normal size, due to multiple

myomas; the tubes and ovaries were essentially normal, save for adhesions about the fimbriated end of the left tube. In attempting to deliver the uterus, after ligating and severing the ovarian vessels and round ligaments, it was torn across at the level of the internal os, due to friability of the uterine wall at this level from the extension of the carcinoma almost to the serosa. The body of the uterus and both tubes and ovaries were removed and radium was applied to the cervical stump. The patient made an uneventful convalescence but eventually died of carcinoma in June, 1933.

A study of the specimen removed showed an advanced papillary adenocarcinoma of the body of the uterus. Carcinoma was not found in the right tube or ovary. Carcinoma having the same histological structure as that of the uterus was present in the fimbriae of the left tube (see Figs. 48, 49 and 50). It was not found elsewhere in the tube. The very superficial character of the growth would apparently exclude a metastasis through the lymph or blood stream. Its histological appearance suggests either an implantation or multicentric origin. Adhesions were found about the fimbriated end of the left tube at the operation, thus suggesting that something had escaped through the ostium of the tube into the peritoneal cavity. This favors the implantation theory.

CASE 9. Adenocarcinoma of the body of the uterus associated with multiple superficial carcinomas of both tubes.

Albany Hospital No. 6707–28. The patient, aged 54 years, complained of uterine bleeding of 7 months duration. At operation, Nov. 3, 1928 (no preliminary curettage), the uterus was found to be of normal size; bilateral hematosalpinx was present, but the ovaries appeared normal. There was no gross evidence of metastases. The appendix, entire uterus and both tubes and ovaries were removed. The patient made a satisfactory convalescence, felt well for several months after the operation, but later died of carcinoma.

On incising the uterus, after its removal, a superficial growth was found replacing the greater portion of the mucosa of the fundus, including that of both cornua. The tubes presented occluded fimbriated ends, dilated lumina and multiple papillary tumors of various sizes scattered over their lining. The data, supporting the theory that the tubal carcinomas primarily could have arisen from the implantation of cancer cells escaping from the uterine tumor through the uterine ostia of the tubes, are presented in Figs. 51 to 58 inclusive.

CASE 10. Multiple superficial papillary adenocarcinomas of the body of the uterus, associated with multiple papillary adenocarcino-

mas of the left tube and a very early squamous cell carcinoma of the vaginal portion of the cervix.

Albany Hospital No. 3760–30. The patient, aged 68 years, complained of uterine bleeding of over a year's duration. At operation, May 29, 1930 (preceded by a preliminary diagnostic curettage), the uterus was found to be of normal size; the left tube was dilated as in hydrosalpinx; the right tube and ovary appeared normal. There was no gross evidence of metastases. The entire uterus, both tubes, ovaries and appendix were removed. The patient made an uneventful recovery and has remained well.

On incising the uterus after its removal multiple superficial papillomas, of different sizes, were found scattered over the mucosa of the body of the uterus, including both cornua and the internal os. They were slightly more numerous in the left side of the body of the uterus and seemed larger in this situation. Similar papillary growths were found scattered over the mucosa of the dilated left tube (Fig. 59), the fimbriated end of which was occluded, but whose isthmus was patent. Carcinoma was not found in the right tube or in either ovary. The data, supporting the theory that the multiple carcinomas of the tubal and uterine mucosae arose from the implantation or grafting of cancer cells on the mucosa of these organs, are furnished in Figs. 59 to 65 inclusive. The possible escape of cancer cells from the uterine growth into the lumen of the left tube, through its uterine ostium, is indicated in Figs. 66 and 67. The reason for the failure of a similar phenomenon in the right tube is indicated in Fig. 68. The presence of a very early squamous cell carcinoma of the cervix (Fig. 69) gives support to the multicentric theory for the pathogenesis of the multiple carcinomas in all the different situations, but does not prove it.

CASE 11. Adenocarcinoma of the right cornu of a markedly bicornuate uterus, associated with multiple adenocarcinomas of the fimbriae and ampulla of the right tube, and carcinoma of the adherent right ovary.

Albany Hospital No. 94611. The patient, aged 56 years, complained of uterine bleeding. At operation, Dec. 27, 1923, (no preliminary diagnostic curettage), a bicornuate uterus was found with marked enlargement of the right cornu. The right tube was distended as in hydrosalpinx and fused by its occluded fimbriated end with the right ovary. The left tube and ovary appeared normal. There was no gross evidence of metastases or extension of the growth beyond the uterus. The appendix, entire uterus, and both tubes and ovaries were removed. The patient made an uneventful recovery but later died of carcinoma.

On incising the uterus a large growth was found confined to the right cornu (see Fig. 70). The microscopic study of sections of the uterus, right tube and ovary (see Figs. 71 to 77 inclusive, with their legends) demonstrated: (*a*) fragments of the carcinoma in the uterine ostium of the right tube; (*b*) carcinoma in newly formed tissue between the right ovary and occluded fimbriated end of the tube, which was fused with the surface of the ovary; (*c*) the invasion of both the ovary and the wall of the tube by carcinoma apparently starting in the opposed surfaces of the two organs; and (*d*) multiple carcinomas of different sizes in the mucosa of the tube. The escape of cancer cells from the uterine tumor, out through the lumen of the tube and its fimbriated extremity, resulting in adhesions between the surface of the ovary and the fimbriated end of the tube, and subsequent infolding of the tubal fimbriae and growth of the carcinoma, could easily account for the pathological findings in this situation. The implantation of carcinoma in the mucosa of the tube would account for the multiple carcinomas, apparently of different ages, found in the ampulla of the tube.

CASE 12. Adenocarcinoma of the body of the uterus, with metastases to the left ovary and bottom of the cul-de-sac, with extension through to the posterior vault of the vagina.

Albany Hospital No. 108718. The patient, aged 50 years, complained of uterine bleeding. At operation, Nov. 21, 1925 (preceded by a diagnostic curettage), the uterus was found to be of normal size and in normal position. The right tube and ovary appeared normal. Two judged endometrial implants were present on the lateral surface of the left ovary. There was an indurated mass in the posterior cul-de-sac which, prior to the operation, was diagnosed as endometriosis with extension through to the posterior vaginal vault. The bottom of the cul-de-sac was obliterated by the fusion of the sigmoid with the posterior wall of the cervix, as often occurs in endometriosis in this situation. There were no other gross evidences of metastases. The appendix, both tubes and ovaries, as well as the entire uterus with the tumor in the cul-de-sac, were removed. After incising the tumor removed from the cul-de-sac it was judged to be carcinoma and not endometriosis. Therefore radium was introduced in the vaginal vault. The patient had an uneventful convalescence, felt well for several months after the operation, but died Aug. 4, 1928, of carcinoma.

An adenocarcinoma of the uterus was found with deep invasion of its wall, but not through to its serosa. The tumor from the cul-de-sac, which was adherent to the posterior surface of the cervix, had the same histological structure as that of the uterine growth, but was not continuous with the latter. The judged endometrial implants of

the left ovary also proved to be adenocarcinoma. The distribution of the growth in the ovary and bottom of the cul-de-sac was similar to that often found in endometriosis. I believe that the carcinoma, in these situations, in this case was secondary to that of the uterus and might have arisen from cancer cells escaping through the lumen of the left tube (see also Figs. 78, 79 and 80 with their legends). Both tubes and the opposite ovary were studied and found free from carcinoma.

CASE 13. Large judged endometrial stromal cell sarcoma of the uterus associated with a superficial papillary adenocarcinoma of the uterine mucosa and a general peritoneal sarcomatosis including the omentum. This case was reported in a previous paper (Case 2 of that paper[4]).

Albany Hospital No. 87700. The patient, aged 54 years, complained of pain in the lower abdomen and uterine bleeding, the latter of over a year's duration. At operation, Dec. 6, 1922 (no preliminary curettage), the greatly thickened omentum was first observed and many peritoneal metastases were noted, as in peritoneal carcinomatosis of ovarian origin. The uterus was enlarged and a portion of its surface studded with metastases. The appendix, entire uterus and both tubes and ovaries were removed. On incising the enlarged uterus a large tumor was found, apparently arising from the mucosa of the anterior uterine wall. It presented the gross appearance of an edematous submucous myoma or sarcoma. The patient reacted badly after the operation and died on the fourth day.

At autopsy a chronic fibrous myocarditis was found; this and the pertitoneal metastases were the only pathological conditions present. Carcinoma in lymph vessels was found only in the uterine cornu, where judged subperitoneal metastases were present (see Figs. 81 and 82 of present paper). The metastases of the ovaries and tubes were of a different type. They were more superficial and were situated in newly formed tissue on the surfaces of these organs (see Figs. 83 to 87 inclusive, with their legends). I believe that these latter metastases could have been of implantation origin from particles of the uterine tumor escaping through the lumina of the tubes into the peritoneal cavity. Fragments of the growth were found in the lumina of the tubes.

CASE 14. Advanced adenocarcinoma of the body of the uterus with deep invasion of the posterior uterine wall, but not through to its serosa, associated with: (*a*) three secondary carcinomas of the

mucosa of the left tube; (*b*) carcinoma of the fimbriae of the right tube; (*c*) general peritoneal carcinomatosis (including the ovaries); and (*d*) massive infiltration of the diaphragm, omentum and tissues of the posterior cul-de-sac with carcinoma. The retroperitoneal lymph nodes were not grossly malignant.

Albany Hospital No. 5493–30. The patient, aged 57 years, presented the physical signs of a peritoneal carcinomatosis of ovarian origin, and such was the preoperative diagnosis. Under spinal anesthesia, July 31, 1930, an exploratory incision was made to ascertain whether or not a major operation was indicated. The patient had difficulty in breathing and in spite of artificial respiration died in 3 hours.

The material for this study was obtained postmortem. The data, indicating that the carcinomas of the tubal mucosa may have resulted from cancer cells escaping into the lumina of the tubes and that the peritoneal carcinomatosis (including the ovaries) primarily arose from cancer cells escaping through the tubes into the peritoneal cavity, are presented in Figs. 88 to 105 inclusive, with their legends.

CASE 15. Adenocarcinoma of the body of the uterus, with peritoneal carcinomatosis restricted to the pelvic structures and the omentum; subsequent metastasis in the scar of the abdominal incision.

Albany Hospital Nos. 111–33, 1617–33 and 4788–33. The patient, aged 67 years, complained of uterine bleeding of 4 weeks duration. She was first admitted to the Albany Hospital, Jan. 5, 1933. A friable, necrotic, cauliflower type of growth was found distending the cervical canal. Biopsy showed it to be an adenocarcinoma of both glandular and solid type. It was impossible at that time to determine whether it began in the mucosa of the cervical canal or had extended down through the cervical canal from the body of the uterus. One hundred milligrams of radium in three capsules, tandem formation, was intruded through the cervix into the uterine cavity. The radium was left in place for 37½ hours. This was associated with a febrile reaction. She was readmitted for a check-up 2 months later. She felt well, her general health having improved greatly. The uterus was freely movable, although induration could be felt in the posterior cul-de-sac. At operation March 3, 1933, metastases were found on the surfaces of both ovaries, the bladder, the vesico-uterine fold, about the right round ligament and in the posterior cul-de-sac. Several epiploical appendages of the sigmoid were fused with the peritoneal metastases in the posterior cul-de-sac. The distribution of the ovarian and peritoneal metastases presented an exact duplication of the lesions often encountered in peritoneal endometriosis. Both tubes appeared patent. The peritoneum of the posterior cul-de-sac in places contained blood pigment, thus suggesting that blood had previously

escaped into the peritoneal cavity. In addition, metastases were present in the omentum. The uterus, both tubes and ovaries were removed. The portion of the omentum containing carcinoma, and all detected peritoneal metastases, were excised. The patient had an uneventful convalescence.

On incising the uterus after its removal the cervical mucosa appeared normal. The uterine mucosa for the most part was smooth and appeared normal, although it was roughened in a few places. Carcinoma was not found in the cervical mucosa, but was present in the roughened areas of the uterine mucosa just mentioned. Carcinoma was not found in the uterine wall (many sections from many blocks) or in the lumina of the tubes. For other evidence, indicating that the metastases to the ovaries and peritoneum of the pelvic structures arose from the implantation of cancer cells escaping through the lumina of the patent tubes during the application of radium, see Figures 106 to 112 inclusive. The patient was examined July 7, 1933, and a small nodule was noted beneath the skin of the scar of the abdominal incision. On bimanual examination marked induration was detected in the posterior cul-de-sac, indicating a further extension of the peritoneal carcinomatosis. The nodule in the abdominal scar was excised and proved to be carcinoma (Fig. 113) of the same histological structure as the primary uterine tumor and that of the peritoneal carcinomatosis observed at operation four months before. The strongest possible circumstantial evidence indicates that this metastasis arose from the successful implantation of particles of cancer in the wound of the abdominal incision at the previous operation.

Discussion

In this study an attempt was made to ascertain the pathogenesis of carcinoma of the tubes and ovaries secondary to that of the body of the uterus, and especially to determine whether or not the transtubal migration of particles of cancer plays an important rôle in the etiology of these secondary tumors. Aside from the purely scientific interest in such a study, it was hoped that information of value might be obtained which could be applied in the treatment of carcinoma of the body of the uterus.

Purposely only those cases of carcinoma of the uterus associated with carcinoma of the tubes or ovaries were chosen to be presented, in which the primary growth was judged to be in the uterus, since those in the tubes and ovaries were less extensive and apparently of

more recent origin. In other cases which I have studied the primary carcinoma was judged to be in the ovary. In still others I was unto able form any opinion of the relation between the growths in the different organs and believed that some of these might possibly be instances of carcinoma of multicentric origin.

Thirteen cases of judged secondary carcinoma of the tubes, ovaries or both organs, of uterine origin, have been presented. Carcinoma was found in both the tube and ovary in six cases, only in the tube or tubes in five and only in the ovary in two. In addition, material from seven other cases of carcinoma of the body of the uterus was utilized because it presented features of importance in the study of the pathogenesis of secondary carcinoma of the tubes and ovaries, of uterine origin.

The following ways may be considered by which carcinoma of the tube may arise from that in the body of the uterus:

1. The continuous extension of carcinoma, arising in or later occupying the uterine cornu, through the uterine ostium of the tube by replacing the tubal mucosa, just as it often replaces the uterine mucosa; this might be designated as surface extension or replacement.

2. The invasion of the wall of the uterine portion of the tube by carcinoma that has invaded the wall of the uterine cornu through which the tube passes.

3. Continuous lymphatic permeation or metastasis through the lymph vessels of the mesosalpinx, and thence in a retrograde course through the tributary lymph vessels of the tube. Probably lymphatic permeation and metastasis also occurs through subperitoneal lymphatics of the uterus and tube, without the passage of the carcinoma through the main lymph channels in the broad ligament.

4. Metastasis through the blood stream.

5. The implantation, on the tubal serosa, of cancer cells which have escaped into the peritoneal cavity either through the lumina of the tubes or from carcinoma that has penetrated the uterine wall and reached the surface of its serosa.

6. The grafting or implantation in the tubal mucosa of cancer cells that have escaped from the uterine tumor into the lumen of the tube.

7. The differentiation of tubal epithelium into carcinoma caused by some agent escaping from the uterine growth into the lumen of the tube.

Carcinoma of the body of the uterus, arising in one cornu or extending to it, frequently passes through the uterine ostium of the tube by surface extension. Instances of this phenomenon are well shown in Figures 27, 28, 29, 30, 54 and 66. Cells, from the growth in this situation, may readily escape into the lumen of the tube, especially if the lumen of its uterine ostium is occluded by the growth (see Figs. 33, 34, 35 and 72). Although I have never observed the continued surface extension of carcinoma of the uterine ostium of the tube out through its entire uterine portion into the isthmus, I believe that it may occur. Blood containing cancer cells also may escape through a patent uterine ostium of the tube from uterine carcinoma not occupying the uterine cornu.

I have not observed the invasion of the wall of the uterine portion of the tube by carcinoma in the wall of the uterine cornu about it in the same manner as the uterine wall itself is invaded, or as structures becoming adherent to the wall of the uterus, at the site of its penetration by carcinoma, are invaded (compare Fig. 9 with Fig. 2).

Lymphatic permeation with or without embolic metastases was responsible for two instances of carcinoma of the tubes, secondary to that of the uterus in this series (see Cases 3 and 4, and Figs. 18, 19, 22, 23 and 24).

In another instance (Case 2) two judged subperitoneal and one submucosal metastasis of the tube were found, which may or may not have arisen from cancer cells carried through the lymph vessels (see Figs. 10, 12 and 13).

I have observed the invasion of the venous sinuses of the uterine wall by carcinoma of the body of the uterus and seen judged cancer emboli in the lumina of these sinuses, but have been unable to determine the part played by the blood stream in the pathogenesis of carcinoma of the tubes and ovaries, secondary to that of the body of the uterus. Possibly some of the metastases shown in Figures 1, 3, 7, 10, 11, 12, 81, 82 and others may have arisen from cancer cells carried through the veins and not the lymphatics. The invasion of the lymphatics about these metastatic tumors does not prove the pathogenesis of the latter. A secondary tumor, irrespective of its pathogenesis, apparently determines its own methods of invasion and dissemination, depending upon its inherent traits, its situation and the structure of the organ in which it is located. The invasion of the venous sinuses of the uterine wall is probably of frequent occurrence in car-

cinoma of the body of the uterus. I believe that the blood stream may play a more important rôle in the etiology of metastases to the tubes and ovaries than I have determined.

Metastases of the serosa of the tubes, as a part of an extensive peritoneal carcinomatosis (sarcomatosis in one instance), was found in two cases (Cases 13 and 14), see Figures 86, 87, 88, 93, 99 and 100. There was strong circumstantial evidence in each instance that the growth first gained access to the peritoneal cavity by the transtubal route.

In the largest and most interesting group (consisting of seven cases, Cases 6, 7, 8, 9, 10, 11 and 14, with carcinoma in both tubes in one case) the carcinoma of the tubes seemingly must have arisen either from a differentiation of tubal epithelium or from cancer cells grafted in the tubal mucosa. In only one case (Case 8) was a single carcinoma found in the tube. In all the others the tumors were not only multiple but varied in size, thus suggesting different ages. They were all superficial. In each instance there was strong evidence that cancer cells could have escaped into the lumen of the tube from the primary uterine tumor, as into the lumen of a lymph vessel. In five of the seven cases the fimbriated end of the tube was occluded. In all of these cases (Cases 6, 7, 9, 10 and 11) carcinoma was present in the uterine cornu of the affected tube (both tubes in one case) with evidence that it had or might have invaded the uterine ostium of the tube (see Figures 47, 54, 55 and 66). Grossly the isthmus of the tube in each case appeared normal. The microscopic examination of the uterine portion of four of these tubes failed to show complete occlusion of any of them, other than that caused by the extension of carcinoma about the uterine ostium into the lumen of the tube. In three instances (Figs. 56, 67 and 72) clumps of cancer cells were found in the lumen of the tube distal to the growth filling the uterine ostium. I believe that the closure of the fimbriated end of the tube, at least in some instances, might have resulted from the development of carcinoma in the tubal mucosa. The possibility of this phenomenon is suggested in Figure 40.

When the fimbriated end of the tube is closed and the uterine ostium plugged with carcinoma a condition of hydro- or hematosalpinx arises. Since the mucosa is stretched under these circumstances injury to its epithelial covering might occur more readily than when the tube is not dilated. In this way conditions more favor-

able for the secondary grafting of cancer cells floating about in the lumen of the tube might be created. These secondary implantations of carcinoma might arise, either from carcinoma established in the tube, or from the repeated escape of cancer cells from the growth situated in its uterine ostium. The data supporting the implantation theory in the above cases are: (*a*) the tubal carcinomas are superficial in character, multiple and of different sizes; (*b*) the evidence that cancer cells from the advancing (and therefore the growing) part of the tumor could well have been discharged into the lumen of the tube; and (*c*) finally the similarity in histological structure of the carcinomas of the tubal mucosa and the implantation carcinomas on the lining of lymph vessels and on the peritoneum. I do not see how the multicentric theory can be applied as logically in the cases just reported, unless the agent causing the differentiation of tubal epithelium into carcinoma is derived from something escaping from the uterine growth into the lumen of the tubes: if it is true here it might also be applied to explain the pathogenesis of similar lymph vessel and peritoneal metastases of accepted implantation origin.

Carcinoma of the ovary was found in eight of the thirteen cases and in only two cases without carcinoma of the accompanying tube. In one instance the growth had invaded the ovary through the utero-ovarian ligament from the continuous extension of the carcinoma that had penetrated the uterine wall (Fig. 14).

In two other instances (Figs. 18 and 25) the ovary was invaded from within by a continuous permeation of the lymph vessels or permeation with metastases. A similar phenomenon was present in the tubes accompanying the ovaries.

In five cases (Cases 11, 12, 13, 14 and 15) the ovarian carcinomas (multiple in each instance) were superficially situated and were either on the surface of the ovary (Figs. 83, 84, 85, 94, 95, 98, 101, 103, 104, 106 and 107) or had apparently invaded the ovary from its surface (Figs. 73, 74, 78 and 80). Two of these were associated with multiple carcinomas of the tubal mucosa (Cases 11 and 14). In one of the latter (Case 11) peritoneal carcinomatosis was not present other than that in adhesions between the surface of the ovary and the occluded fimbriated end of the tube. In the other there was an extensive peritoneal carcinomatosis. In another case (Case 13) an extensive peritoneal sarcomatosis was present with implantations on the serosa of the tube, but without metastases to the tubal mucosa.

I believe that the transtubal migration of cancer cells from a uterine growth played a very important rôle in the etiology of the cases of judged secondary carcinoma of the tube or ovaries, or both organs, just reported, namely, in nine of the thirteen cases. I do not desire to create the impression that the transtubal migration of cancer cells in either direction necessarily plays as important a rôle in the pathogenesis of all cases of carcinoma in these different organs as it did in the cases just reported.

In two cases of this series (Figs. 37 and 59) judged implantation of carcinoma in the endometrium is described secondary to a primary carcinoma of the body of the uterus. I believe that this is of rare occurrence. I have been looking for it since I encountered Case 10, three years ago, and have encountered only one other instance of multiple carcinomas of the uterine mucosa and that is shown in Figure 37.

In Figure 39 is shown a judged implantation of uterine carcinoma on the mucosa of the cervix. It is the only instance of the kind that I have found, and I have been looking for it ever since I encountered this one ten years ago. I have been greatly impressed with the rarity of the involvement of the cervical mucosa in carcinoma of the body of the uterus, even in advanced cases.

In Figure 36 is shown a judged implantation of uterine carcinoma in the scar of a vaginal wound. On the other hand, the pathogenesis of the vaginal metastasis shown in Figure 38 is more difficult to state. I have been greatly interested in the etiology of the vaginal metastases found in carcinoma of the body of the uterus, but have been unable to make a satisfactory study of this condition. The majority of patients with carcinoma of the body of the uterus are past the menopause. Senile changes in the vaginal mucosa, often with superficial ulcerations, are of frequent occurrence in women of this age. It occurred to me that these superficial ulcerations might furnish favorable soil for the grafting of any viable cancer cells escaping through the cervical canal. In spite of my enthusiasm over the implantation of carcinoma, it has been my impression that in the majority of the instances of vaginal metastases that I have encountered the lymphatic or venous route offered a more rational explanation of their pathogenesis. This is particularly true when additional metastases are found elsewhere than in the vagina.

An instance of metastasis in the scar of the abdominal incision was

encountered in this series. The circumstantial evidence leading to the development of this metastasis indicates that it arose from the implantation of cancer cells during the operative removal of the uterus, tubes and ovaries and the excision of peritoneal implantations four months before. At the time of the operation there were no visible metastases in the peritoneum of the abdominal wall (see Case 15, and Fig. 113). This is another contribution to the implantation of carcinoma. The fundamental stages in this process are the same in all instances, namely, the lodging of cancer cells in a wound, however created, with the attempted healing of the wound and the continued growth of the cancer cells in their new situation.

The conditions found in this small series of cases supports the stand taken by those who believe that carcinoma of the body of the uterus, when feasible, should be treated by the removal of the entire uterus and *both tubes and ovaries*. It condemns the routine treatment of carcinoma of the body of the uterus with radium, since it cannot be expected to reach the possible secondary cancers in the tubes and ovaries. Also the introduction of radium into the uterine cavity is associated with the danger of disseminating blood containing particles of cancer through patent tubes into the peritoneal cavity (see Case 15, and Figs. 106 to 112). Radium should be used only in poor operative risks, or after the uterus has been removed.

The operative removal of advanced carcinoma of the body of the uterus is associated with a relatively high primary mortality and a low percentage of cures, yet three of these patients with secondary carcinoma in the tubes are living and clinically free from cancer — Case 6 after ten years, Case 7 after four years and Case 10 after three years.

Conclusions

By continuous extension, carcinoma of the body of the uterus invades the potential tissue spaces of the uterine wall and may even penetrate the entire wall, including its serosa. Pelvic structures frequently become adherent to the surface of the uterus thus penetrated by the growth and in turn are invaded by it. Circumstantial evidence indicates that cancer cells may escape into the peritoneal cavity from a growth that has reached the surface of the uterus before it became adherent to nearby structures, and may give rise to implantation peritoneal carcinomatosis.

Both the lymphatics and venous sinuses of the uterus are invaded by the continuous extension of carcinoma. Secondary carcinoma of the tubes and ovaries arises from the continuous permeation of the lymphatics and also from cancer emboli. Circumstantial evidence indicates that cancer cells become implanted on the endothelial surface of lymphatics just as similar evidence indicates that cancer cells escaping into the peritoneal cavity, from ovarian carcinoma, become implanted on the surface of the peritoneum.

By the continuous extension of carcinoma of the body of the uterus the mucosa is replaced by the growth, and when situated in a uterine cornu it frequently extends through the uterine ostium of the tube and replaces the mucosa of the uterine portion of the tube. Particles of cancer are broken off from the growing (advancing) portion of the growth in this situation and as emboli migrate into the lumen of the tube beyond, as similar emboli migrate in lymph vessels and into the peritoneal cavity. Superficial carcinomas are found in the mucosa of the tubes, in their fimbriae, and on the ovary and peritoneum of patients with carcinoma of the body of the uterus. They present the same histological structure as recognized implantation carcinoma of the lining of lymphatics, and also on the peritoneum from ovarian carcinoma, with evidence that particles of cancer from the primary uterine growth could have reached the situation of these metastases through the lumina of the tubes. Therefore, I believe that their pathogenesis in many instances is the same; namely the lodging of the cancer cells on the surface of their host, injury to that surface already present or created by the carcinoma, the attempted repair of the injury with the continued growth of the carcinoma in this situation. In like manner particles of uterine carcinoma become implanted in the uterine and cervical mucosa, and in wounds of the vagina and of abdominal incisions.

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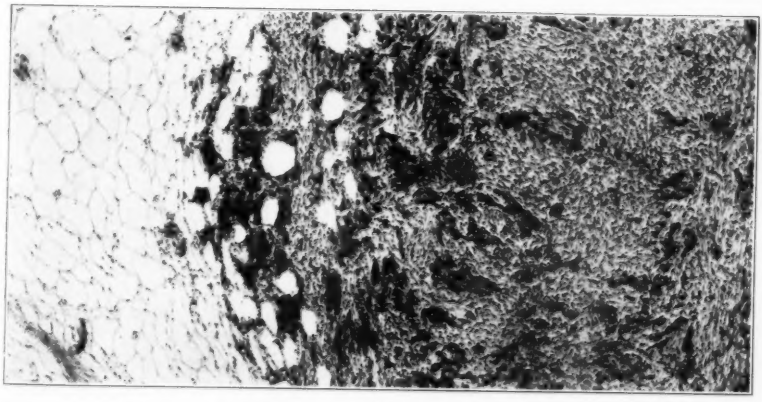
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Photomicrograph of a sagittal section of a portion of the left uterine cornu (Case 1). It shows well the invasion of the posterior wall of the uterus by the carcinoma. Here the growth is an adenocarcinoma with the solid phase predominating (see Fig. 5). The mucosa lining the anterior wall of the uterine cavity has been replaced by a papillary adenocarcinoma, without evidence of invasion of the uterine musculature. The variations in the histological structure of the growth in different portions of this specimen demonstrate the difficulty that may arise in typing adenocarcinoma of the uterus. A judged subperitoneal metastasis is indicated at "a" and an embolus of cancer cells in a lymphatic at "b." Many sections studied of this portion of the block failed to demonstrate that the carcinoma in the lymphatic "b" was continuous with the primary growth. Several other subperitoneal metastases were present in the left uterine cornu; for a higher magnification of one of these see Fig. 3. × 5.

FIG. 2. Photomicrograph of a portion of the posterior uterine wall and the omentum that is fused with it (Case 1). The strands of carcinoma shown in this section are continuous with the primary growth indicated in the preceding illustration, and have penetrated the entire thickness of the posterior uterine wall. Cancer cells may have escaped into the peritoneal cavity before the omentum came to the rescue, the latter preventing the dissemination of cancer cells, just as it often checks the spread of bacterial infections. The omentum, (to the left) adherent to the posterior surface of the uterus, has been invaded by the growth. × 54.

1



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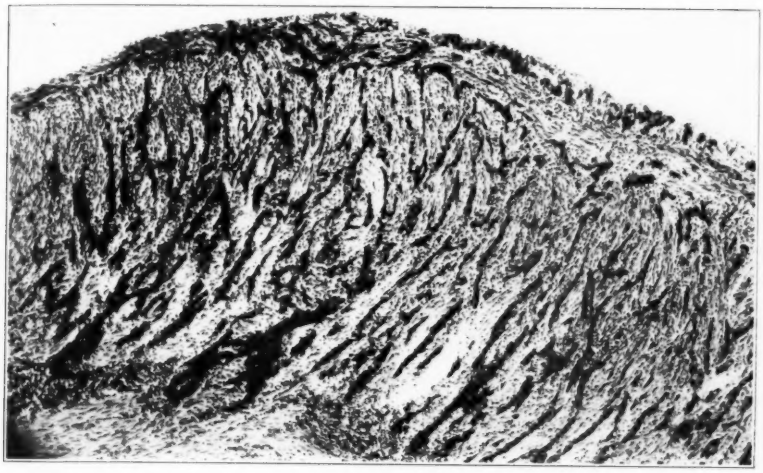
Sampson Carcinoma of Tubes and Ovaries

Plate 2

Fig. 3. Photomicrograph of a section through a judged subperitoneal metastasis of the left uterine cornu, similar to the one shown in Fig. 1, but smaller. To the left the carcinoma has not reached the peritoneum, but in the center and to the right it has. The condition present in the latter situations suggests that cancer cells might have escaped into the peritoneal cavity (see next illustration). It was impossible to determine the pathogenesis of this metastasis and the one shown in Fig. 1, as well as other similar ones present in this specimen. They were judged to be of lymphatic origin, the cancer cells having been deposited in the subperitoneal tissues with subsequent extension toward its surface. × 54.

Fig. 4. Photomicrograph of a section of the sediment from centrifugalized blood-stained fluid obtained from the posterior cul-de-sac at the time of the operation. I believe that the large mass of faintly staining cells, to the left, is of mesothelial origin. In the center are clumps of hyperchromatic cells with variation in the size and staining qualities of their nuclei. These cells resemble those of the primary growth (see next illustration) more closely than they do the judged mesothelial cells. Clumps of these cells, to the right, are arranged in the form of glands. Mitotic figures are present. I am unable to assert that they are clumps of cancer cells and not differentiated mesothelial cells. If cancer cells they may have escaped from strands of carcinoma extending into the peritoneal cavity, from the direct extension of the growth through the uterine wall as suggested in Fig. 2, or from a similar phenomenon indicated in Fig. 3. On the other hand they may have escaped through the lumina of the tubes during the diagnostic curettage 6 days before. × 130.

Fig. 5. Photomicrograph of the primary uterine tumor taken from the portion of the growth invading the posterior uterine wall, Fig. 1. It is an adenocarcinoma — solid phase predominating. The masses of cancer cells and the gland in the center closely resemble the clumps of hyperchromatic cells and the cells with glandular formation found in the sediment obtained from fluid in the posterior cul-de-sac shown in the preceding illustration. × 130.



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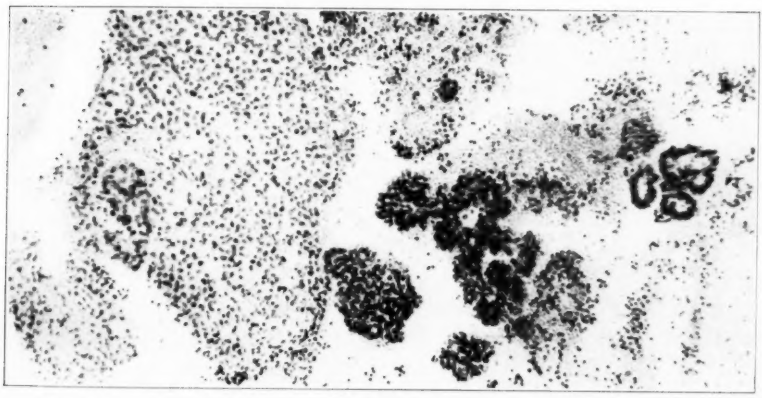

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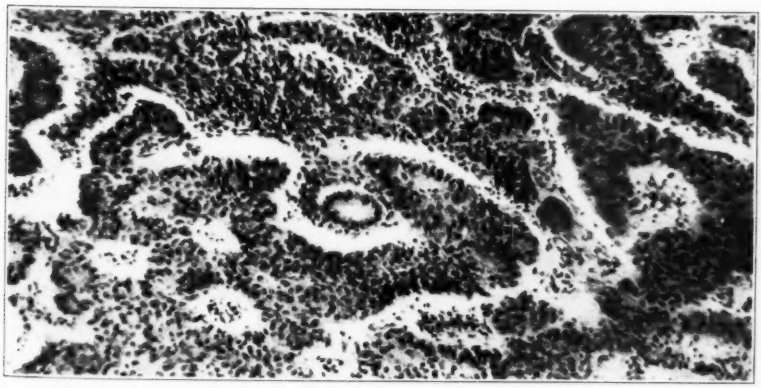

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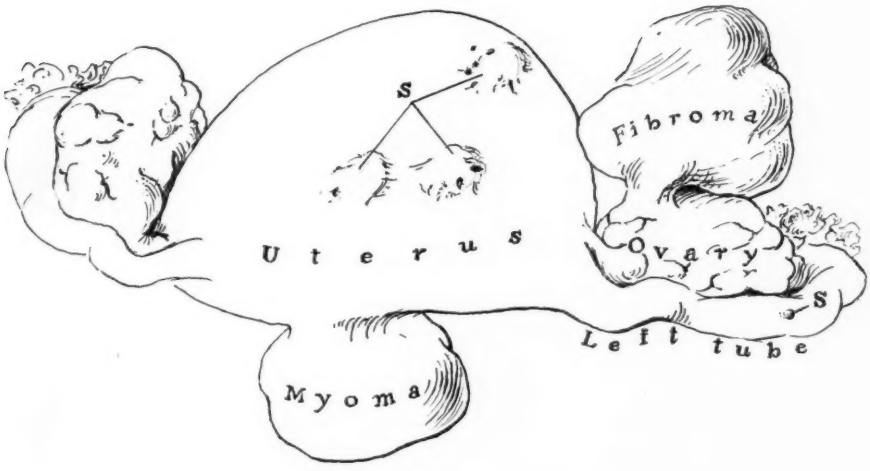
Sampson Carcinoma of Tubes and Ovaries

PLATE 3

FIG. 6. Fundus of the uterus with the tubes and ovaries (Case 2). The uterus contained multiple small myomas and was adherent posteriorly to the sigmoid and the terminal loop of the ileum, due to the extension of the growth through the posterior wall of the uterus. Peritoneal carcinomatosis was also present in the posterior cul-de-sac. Slightly elevated scars (s) are present on the surface of the uterus grossly (and microscopically) similar to the subperitoneal metastases in the left uterine cornu of the preceding case. The right tube and ovary are normal. A pedunculated fibroma is attached to the posterior surface of the left ovary, which has been invaded by the uterine carcinoma (see Fig. 14). The left uterine cornu is larger than the right, due to its infiltration with carcinoma (see Fig. 9). Two subperitoneal metastases are present in the ampulla of the left tube (one is shown at "s"). See also Figs. 10 and 12. Natural size.

FIG. 7. Photomicrograph of a cross-section of a portion of the posterior uterine wall through one of the scars shown in the preceding illustration. The uterine wall has been infiltrated by the direct extension of the primary growth (lower third of the photomicrograph). An intramural myoma is present — carcinoma was not found in it. A judged subperitoneal metastasis is indicated at "S" similar to the one shown in Fig. 1. Serial sections of this block were not made and therefore the origin of the growth in this situation by continuous extension from the primary tumor cannot be excluded absolutely. × 5.

FIG. 8. Photomicrograph of a portion of the uterine wall infiltrated by the carcinoma. It is an adenocarcinoma with both glandular and solid arrangement of the cancer cells. × 54.



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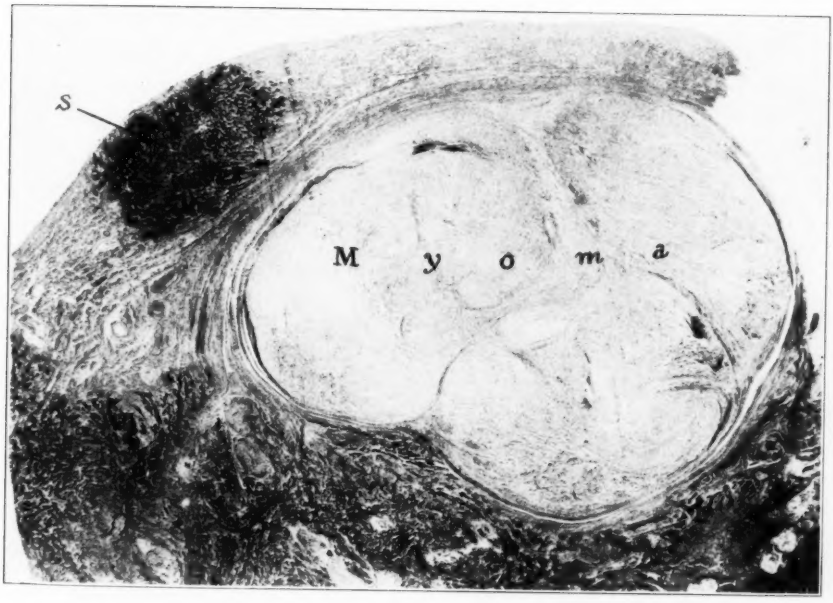

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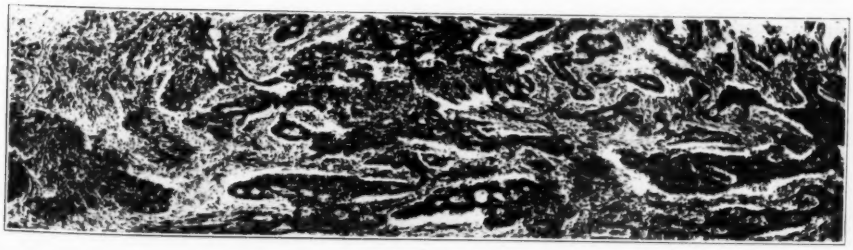

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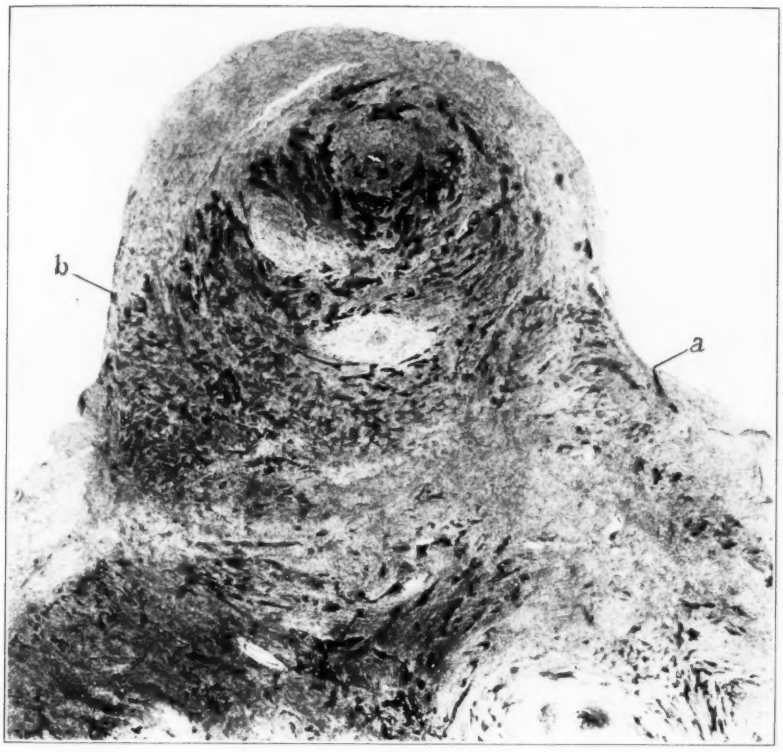
Plate 4

Fig. 9. Photomicrograph of a section through the left cornu of the uterus. The uterine cornu is infiltrated by the continuous extension of the carcinoma. The tube, shown in cross-section, is surrounded by the growth, but is not actually invaded by it. Its lumen is patent and is lined by normal mucosa. At "a" and "b" the strands of carcinoma have extended through the peritoneum. × 10.

Fig. 10. Photomicrograph of a portion of two longitudinal sections (from different levels of the same block) of the distal end of the left tube. At "b" is a judged subperitoneal metastasis similar to the one shown in Fig. 7 (compare also with Figs. 11 and 12). At "a" is a judged submucosal metastasis in the fimbriae of the tube. Compare with Figs. 11, 12 and 13. × 5.



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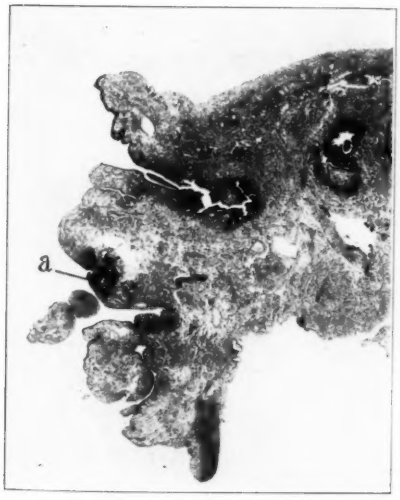


10

Sampson

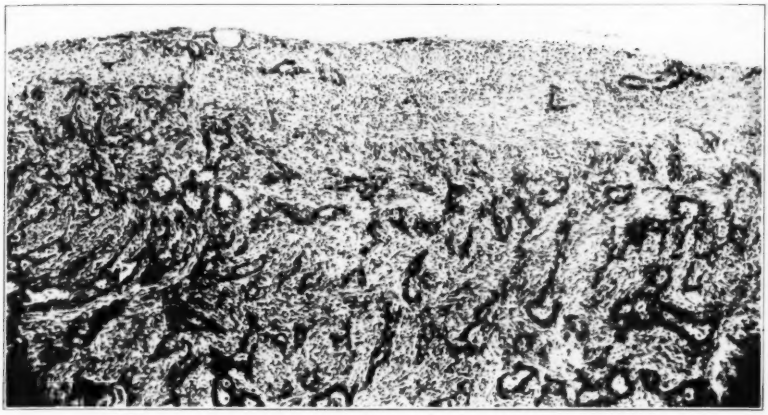
Carcinoma of Tubes and Ovaries

PLATE 5

FIG. 11. Photomicrograph of a superficial portion of the judged subperitoneal metastases of the uterine wall shown in Fig. 7. The growth apparently started in the uterine wall and has extended through to the peritoneal surface. Compare with Fig. 3. In this situation the glandular phase of the carcinoma predominates. × 54.

FIG. 12. Photomicrograph of the judged subperitoneal metastasis to the tubal wall indicated by "b" of Fig. 10. The general histological formation of this tumor is similar to that of the preceding one. It seems to me that the growth probably started in the deeper portion of the subperitoneal tissues and spread in all directions, even to the surface of the peritoneum. Others may claim that it arose from an activation and differentiation of the mesothelium (see arrow) or the implantation of cancer cells on the peritoneal surface of the tube. Still others may assert that the apparent extension of the carcinoma to the surface of the peritoneum is misleading, that what appears to be a direct extension of the growth to the surface of the peritoneum is in reality the downward growth of the activated mesothelium to meet the carcinoma. A careful study of the peripheral extensions of the growth shows that they are the same in all directions. × 54.

FIG. 13. Photomicrograph of the judged submucosal metastasis of the fimbriae of the tube shown in "a" of Fig. 10. If the growths shown in the preceding two illustrations are subperitoneal metastases why should not this one be a submucosal metastasis rather than a primary tumor in this situation, or an implantation? Its apparent origin from the epithelium (see arrow) may be misleading. In reality it may be the extension of the growth to the mucosal surface of the fimbriae as the carcinoma in the two preceding illustrations extended to the surface of the peritoneum. It was impossible to ascertain whether the cancer cells reached the situations indicated in these three metastases through the lymphatics or veins. With our present knowledge of the pathogenesis of similar metastases we believe that the lymphatics are the most likely route. × 54.



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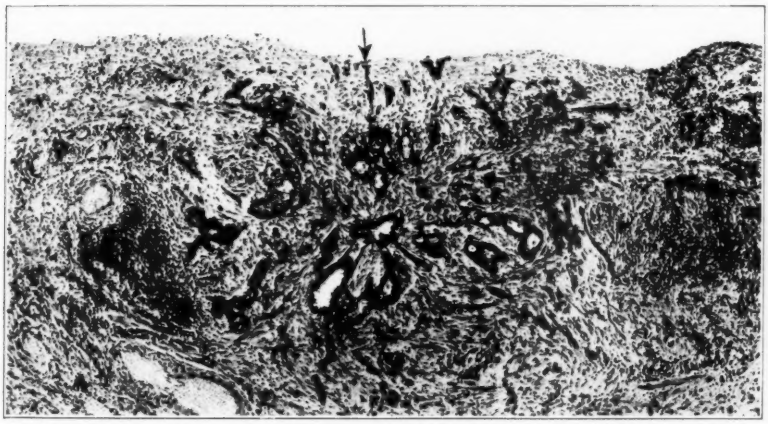

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Sampson Carcinoma of Tubes and Ovaries

PLATE 6

FIG. 14. Photomicrograph of a section of the left ovary, the utero-ovarian ligament and a portion of the wall of the uterus (Case 2). By a continuous extension the carcinoma has penetrated the uterine wall, passed through the utero-ovarian ligament (see arrows) and invaded the ovary. At "a" the growth has entered the lumen of a vessel (see the next illustration). The continuous invasion of the ovary was demonstrated by serial sections of this portion of the block. At "b" the growth has extended through the uterine wall to the peritoneal cavity (see Figs. 16 and 17), just as it invaded the lumen of the vessel at "a." × 8.

14

Sampson Carcinoma of Tubes and Ovaries

PLATE 7

FIG. 15. Photomicrograph (higher magnification) of the area "a" of the preceding illustration demonstrating the extension of the carcinoma into the lumen of a vessel. It resembles a lymph vessel more than a vein, but a small amount of blood was present in its lumen. One can readily see that cancer cells could escape easily from the growth which has penetrated the lumen of the vessel and as emboli might be carried wherever the blood or lymph stream might take them. In this section the glandular phase of the carcinoma predominates. × 54.

FIG. 16. Photomicrograph (higher magnification) of area "b" of Fig. 14. There has been a peritoneal reaction to some irritant with the development of newly formed tissue on the surface of the peritoneum. Enmeshed in this tissue are clumps of cells ("a," "a" and "a") histologically indistinguishable from the solid phase of the primary uterine growth, shown in Fig. 8, and also in the uterine wall beneath the newly formed tissue ("b" and "b"). Serial sections were made of this portion of the block to determine the relation between the carcinoma in the uterine wall and the clumps of judged cancer cells in the newly formed tissue above it. See the next illustration. × 54.

FIG. 17. Photomicrograph of a section from the same series as the one shown in the preceding illustration. The continuous extension of the carcinoma (see arrow) through the uterine wall to the surface of the peritoneum is well shown. In this section the solid phase of the tumor (shown in Fig. 8) prevails; the reaction of the peritoneum, attempting to check the further extension of the growth, is indicated by the newly formed tissue on the surface of the uterus, also shown in the preceding illustration. The clumps of judged cancer cells ("a" and "a") enmeshed in this newly formed tissue are not continuous with the cancer ("b" and "b"), which has penetrated the uterine wall. We have in this, and other sections from this block, the strongest possible circumstantial evidence that strands of carcinoma penetrating the uterine wall may give rise to implantation carcinoma of the peritoneum about the site of the penetration of the uterine wall by the growth. If here, why not also in the peritoneum of the posterior cul-de-sac where a peritoneal carcinomatosis was present? × 54.

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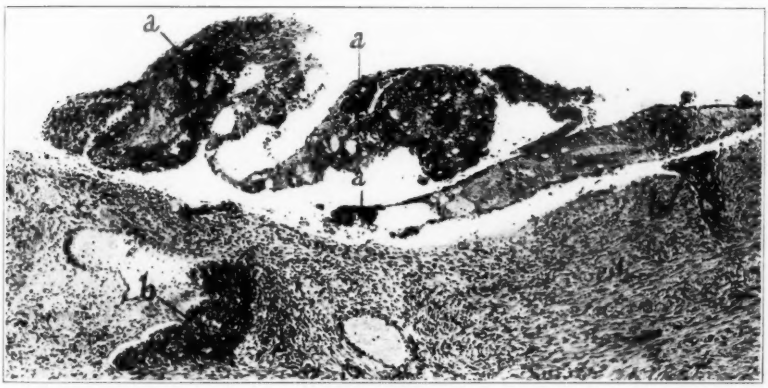

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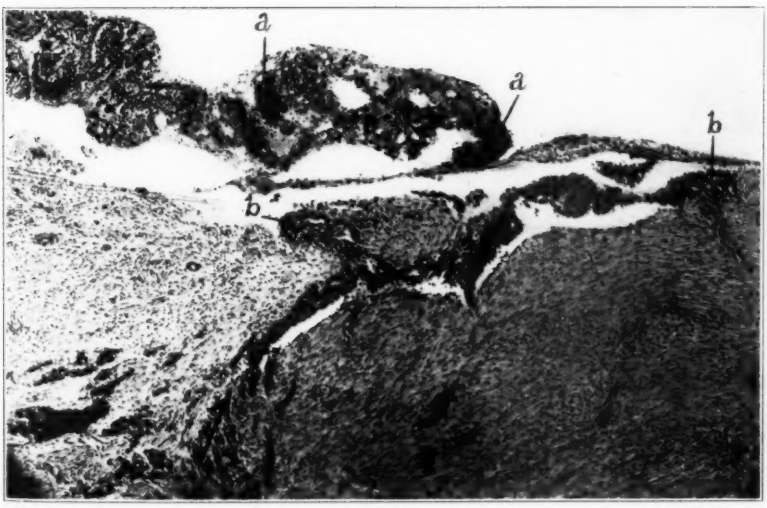

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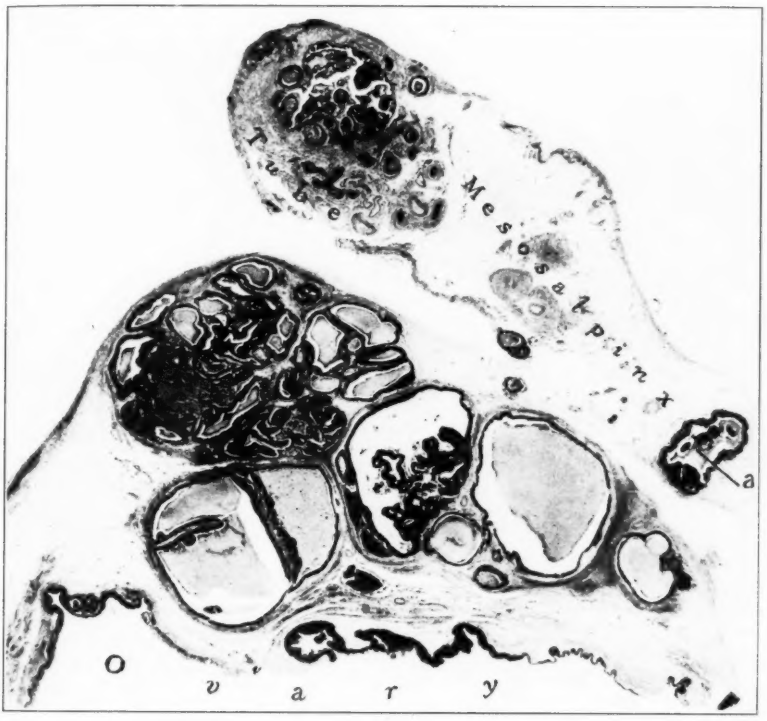
PLATE 8

FIG. 18. Photomicrograph of a cross-section of the right tube, mesosalpinx and portion of the enlarged ovary from a patient with extensive carcinoma of the body of the uterus, secondary to that of the cervix (Case 3). In the gross specimen one could follow easily the subperitoneal lymphatics distended with cancer cells as with a white injection mass, an excellent example of lymphatic permeation. Note a large lymphatic "a" in the mesosalpinx lined with carcinoma. The lymphatics of the tube are also distended with carcinoma, most marked in the mucosa (see also the next illustration). The carcinoma of the ovary tends to form cysts. In both organs the growth has developed within the organ and bears no relation to their surfaces. One would not be tempted to attribute the conditions shown here to the transtubal migration of cancer cells. × 5.

FIG. 19. Photomicrograph (higher magnification) of a portion of the mucosa of the tube shown in the preceding illustration. The lymphatics of the mucosal folds are distended with carcinoma. In one place "a" the growth has extended through the wall of a lymphatic and invaded the lumen of the tube. Its relation to tubal epithelium is well shown to the right of the letter "a." In some ways it, here, suggests that the growth might have arisen from the tubal epithelium. A fragment of carcinoma lying free in the lumen of the tube is indicated at "b." Such a fragment might escape into the peritoneal cavity. The conditions presented in these photomicrographs admit of only one rational interpretation, and that is that the carcinoma of the tube and ovary is secondary to that of the uterus by lymphatic permeation, or metastases, or both phenomena. × 54.



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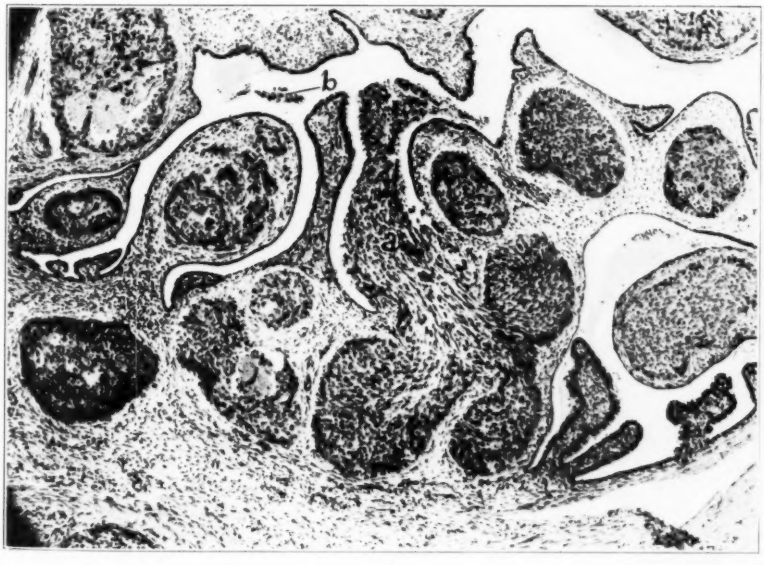

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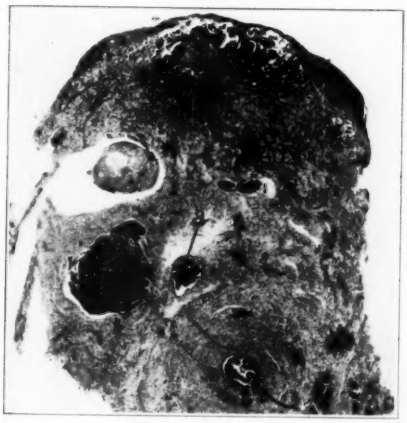
PLATE 9

FIG. 20. Photomicrograph of a section of the distal portion of the left uterine cornu, including the tube (Case 4). The uterine wall is infiltrated with carcinoma. The lumen of the tube is patent, but carcinoma is present in its mucosa, beneath the epithelium. At "a" an apparent implantation of carcinoma is shown on the lining of a lymph vessel. See next illustration. × 10.

FIG. 21. Photomicrograph (higher magnification) of the lymph vessel indicated by "a" of the preceding illustration. When this implantation was found all sections of the block, which had been cut already, were stained and serial sections were then made of the rest of the block. We were unable to demonstrate that this apparent implant was continuous with carcinoma elsewhere. I believe that it is an implantation of carcinoma of the skin graft type occurring on the endothelial surface of a dilated lymph vessel, just as an ovarian carcinoma sometimes becomes implanted on the peritoneum. × 54.

FIG. 22. Photomicrograph of a cross-section of the tube (Case 4). The patent lumen of the tube appears in the lower right portion of the photomicrograph. The wall of the tube is greatly thickened, due to lymphatic stasis, the result probably of the occlusion of efferent lymph vessels by carcinoma. Some of the dilated lymph vessels are almost completely filled with the growth, others are not. In places the carcinoma is attached to the wall of the lymph vessels, but in the majority of the lymph vessels the growth appears as clumps of cells (emboli) floating about in the lumen of the vessel or as cross-sections of strands of carcinoma. Serial sections were made to determine whether or not true emboli were present. We were able to demonstrate that at least some of the carcinoma in these dilated lymph vessels are true emboli and not long strands or threads (roots) continuous with the primary tumor. × 10.

FIG. 23. Photomicrograph of a section of a dilated lymph vessel in the mesosalpinx. True emboli are present. In the center of the photomicrograph is an implantation of the foreign body type, such as often occurs in the implantation of ovarian carcinoma on the peritoneum. Compare with Fig. 21. × 54.



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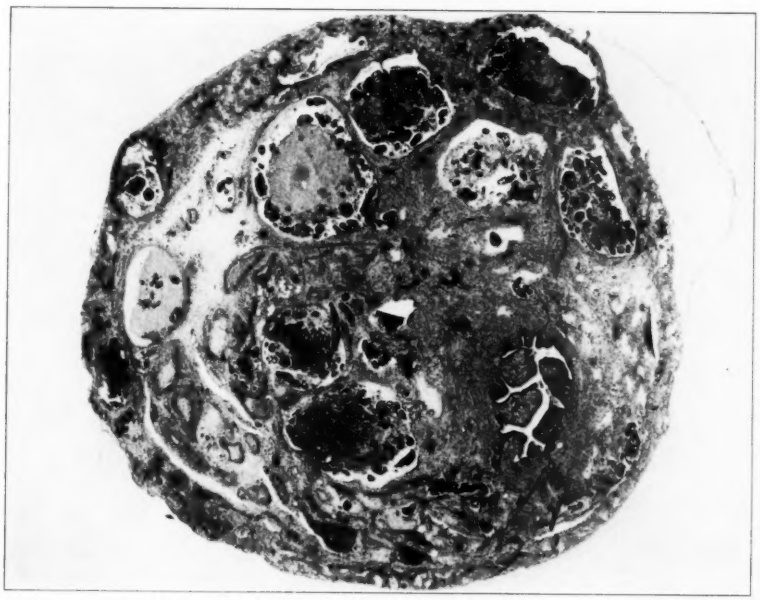

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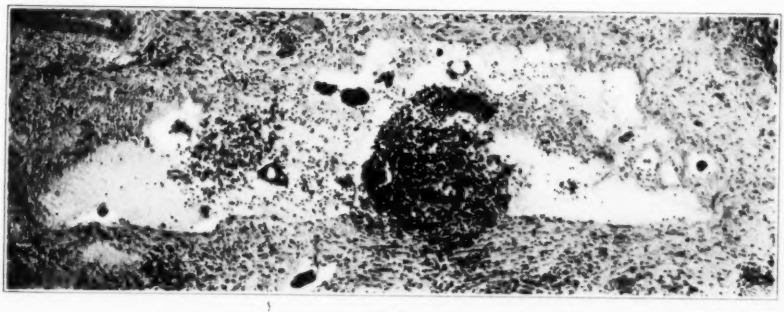

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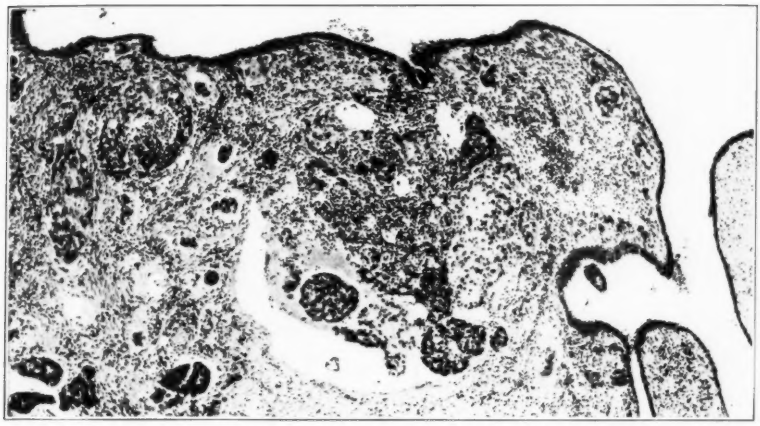
Plate 10

Fig. 24. Photomicrograph of a section of the tubal mucosa (Case 4) demonstrating the pathogenesis of carcinoma of this tube from carcinoma of the uterus, as a result of lymphatic permeation and metastasis (compare with Fig. 10). × 54.

Fig. 25. Photomicrograph of a section of the ovary (Case 4), demonstrating the invasion of the ovary by carcinoma from that of the uterus through the lymphatics in the hilum of the ovary. × 5.

Fig. 26. Photomicrograph of a section through the cortex of the ovary demonstrating the invasion of this portion of the ovary from carcinoma entering the ovary through its hilum and not its surface. × 54.



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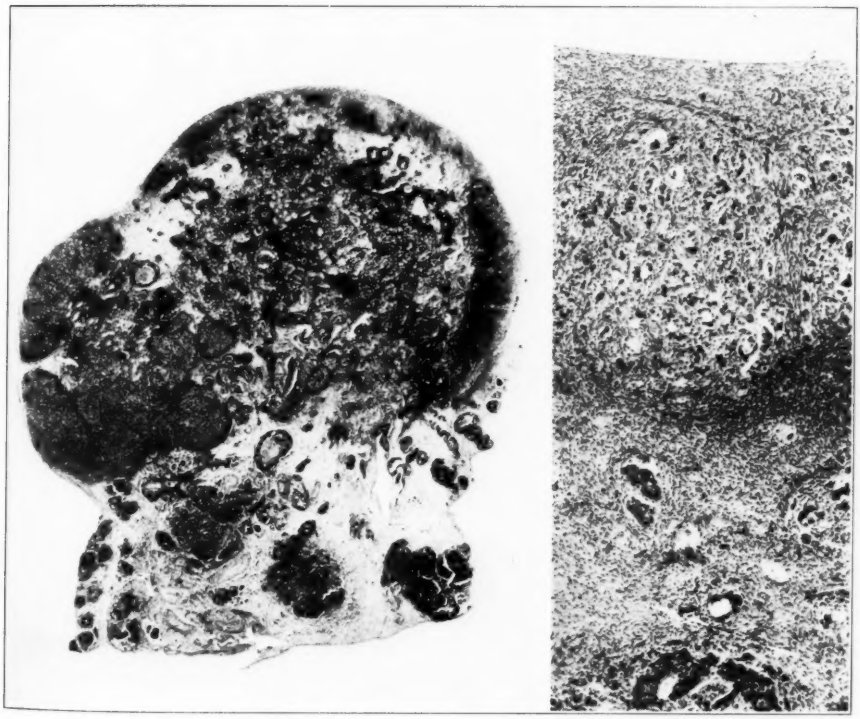

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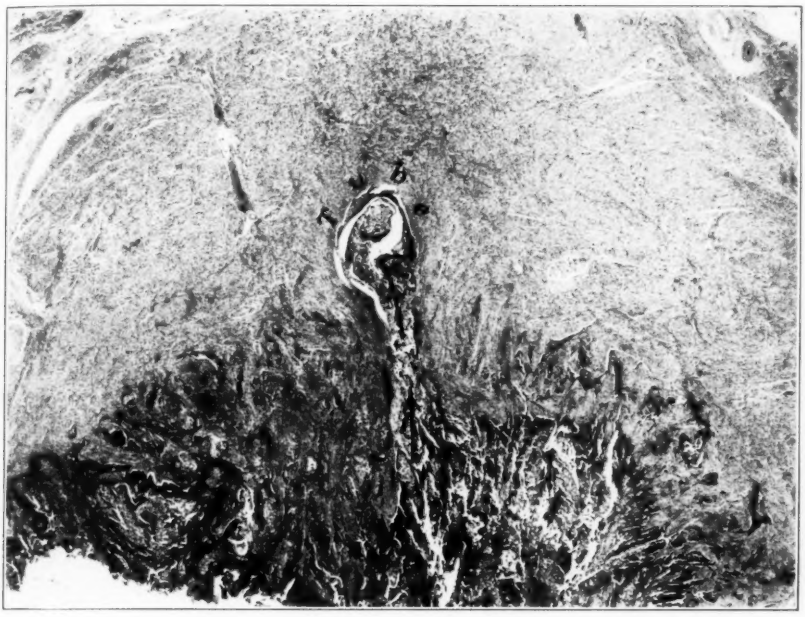
PLATE 11

FIG. 27. Photomicrograph of a section (horizontal plane) of a portion of the uterine cornu including the uterine ostium of the tube from a patient aged 63 (Albany Hospital No. 8868–32), with adenocarcinoma of the body of the uterus involving the entire uterine mucosa. The patient had a previous operation 6 years before, when the pelvic floor was repaired, the appendix and both tubes and ovaries removed, and the uterus fixed to the abdominal wall. By continuous extension the carcinoma has invaded the uterine wall; and by a similar process it has passed through the uterine ostium of the tube into the uterine portion of the tube, replacing the mucosa of the latter. One can realize that particles of carcinoma might have broken off from the growth in the tube and might have migrated into the lumen of the tube beyond, had the tube not been removed at a previous operation. A similar condition was present in the opposite uterine cornu. × 10.

FIG. 28. Photomicrograph of a section (vertical plane) of the uterine cornu, including the uterine portion of the tube from a patient aged 59 (Albany Hospital No. 8521–30), with an advanced adenocarcinoma of the body of the uterus. By continuous extension the carcinoma has invaded the uterine wall, penetrated the lymphatics, and also extended through the uterine ostium of the tube into the uterine portion of the latter. A similar condition was present in the opposite uterine cornu. Bilateral hydrosalpinx, evidently due to a previous pelvic infection was present. Both tubes were occluded in their uterine portion. × 10.



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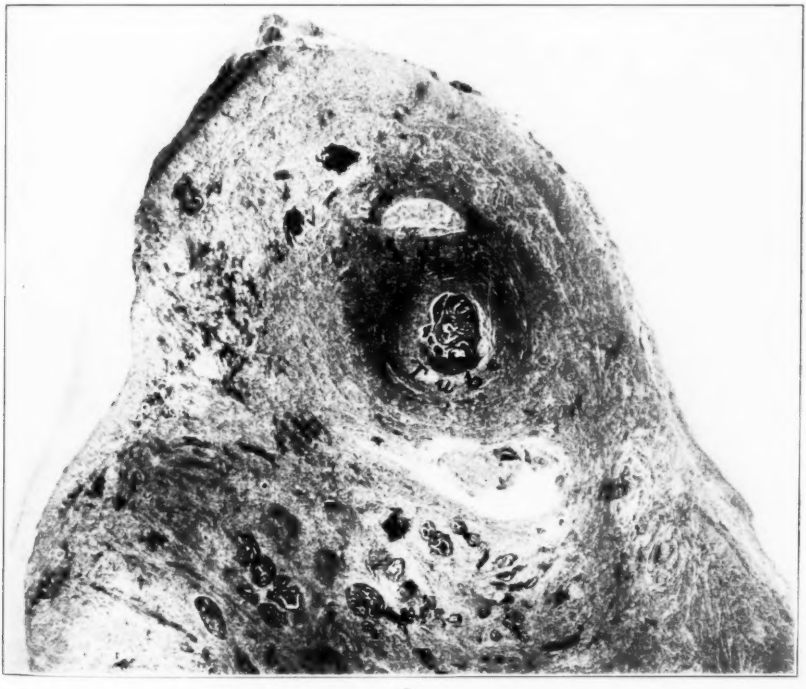

28

Sampson Carcinoma of Tubes and Ovaries

PLATE 12

FIG. 29. Photomicrograph of a longitudinal section (vertical plane) of the left uterine cornu, including the uterine portion of the tube and a part of its isthmus, from a patient aged 56 (Albany Hospital No. 62–33), with a very early adenocarcinoma of the body of the uterus confined to the mucosa of the left uterine cornu. By continuous extension (shown by serial sections) the carcinoma has extended through the uterine ostium of the tube into the uterine portion of the latter, just as it spreads by replacing the mucosa of the body of the uterus. Carcinoma was not found in the tube beyond the place indicated in the photomicrograph. × 5.

FIGS. 30, 31 and 32. Photomicrograph from a series of cross-sections of the uterine cornu and isthmus of the tube from a patient aged 64 (Albany Hospital No. 7587–32), with an advanced adenocarcinoma of the body of the uterus replacing the entire endometrium and invading the uterine wall. By continuous extension the carcinoma has passed through the uterine ostium of the tube into the uterine portion of the latter (Figs. 30 and 31). A cancer embolus is present in the lumen of the isthmus (Fig. 32); this is not continuous with the growth shown in the preceding photomicrograph. × 10.

FIG. 33. Photomicrograph (higher magnification) of the carcinoma shown in Fig. 30. Its histological structure suggests a primary adenocarcinoma of the tubal mucosa. In reality it represents a graft of the uterine carcinoma in the tubal mucosa from the continuous extension of the primary tumor through the uterine ostium of the tube. × 54.

FIG. 34. Photomicrograph (higher magnification) of the carcinoma shown in Fig. 31. It appears to be lying free in the lumen of the tube. In reality it is continuous with the growth shown in the preceding photomicrograph. It represents the outgrowths of the latter into the lumen of the tube as carcinoma grows into the lumen of a vein or lymph vessel. It is conceivable that emboli might arise from such a process and be carried to other portions of the tube, or even escape through the fimbriated end into the peritoneal cavity. Such emboli were found in the isthmus (Fig. 32), and likewise in the ampulla of the tube (see insert "a" and also Fig. 35). The embolus shown in insert "a" was in the ampulla of the tube about 1.5 cm. from the abdominal ostium. While some of the cells stained as well as those in the growth from which it was apparently derived, others did not. Karyorrhexis of some of the nuclei is present. × 130.

FIG. 35. Photomicrograph of a portion of a section of the ampulla of the same tube shown in the preceding illustration. Another cancer embolus is shown situated between the folds of the tubal mucosa. The cells in this embolus stained better than those shown in the embolus of insert "a" of Fig. 34. Circumstantial evidence suggests that these emboli were present in the tube prior to the operation. Many interesting questions arise. Do these emboli wandering about in the lumen of the tube increase in size? Compare them with the smaller outgrowths shown in Fig. 34. How long will the cancer cells live in these emboli? Can they become grafted in the tubal mucosa if a suitable soil is found or created? See Fig. 42. What might happen if they escaped into the peritoneal cavity? × 130.

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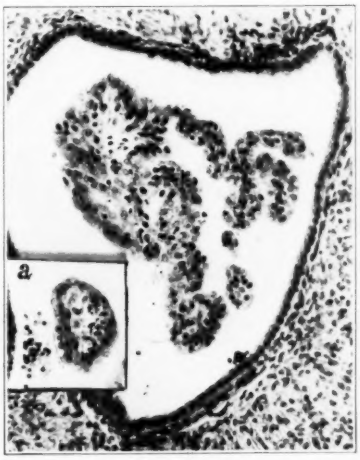

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35

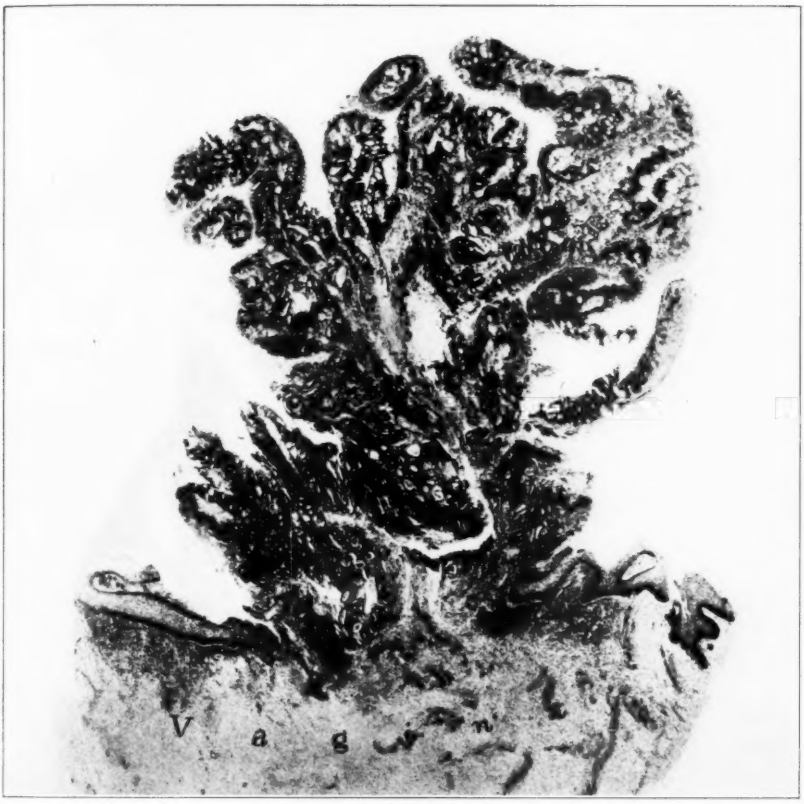
Sampson

Carcinoma of Tubes and Ovaries

PLATE 13

FIG. 36. Photomicrograph of a section of a metastasis of a papillary adenocarcinoma of the uterus to the vagina (Case 5). Circumstantial evidence indicates that it is a wound implantation. The hymen was injured in exposing the cervix while obtaining a biopsy prior to the introduction of radium 2 months before. The papillary carcinoma, with the same histological structure as the primary tumor, has arisen in the scar of this wound. Note the superficial character of the tumor and the normal stratified epithelium on both sides of its base. Compare also with Fig. 21, demonstrating a similar papillary carcinoma implanted on the surface of a lymph vessel. The various stages in the development of the implants in the two situations are the same, namely, the grafting of cancer cells on or in a wound with the attempted healing of the wound and the continued growth of the cancer cells in this situation. This was the only vaginal metastasis found. × 15.

FIG. 37. Photomicrograph of a possible implantation carcinoma in the uterine mucosa secondary to adenocarcinoma of the uterus from a patient aged 57 (Albany Hospital No. 5221–29). On incising the uterus, after its removal, this papillary growth was noted near the primary tumor but grossly not continuous with the latter. Serial sections failed to demonstrate any continuity between the primary uterine tumor and the small papillary growth near it. Although a multicentric origin cannot be excluded, the possibility of an implantation metastasis must be considered. Since cancer cells can become implanted on endothelial surfaces and in vaginal wounds why not in uterine mucosa, if a suitable soil be created? × 8.



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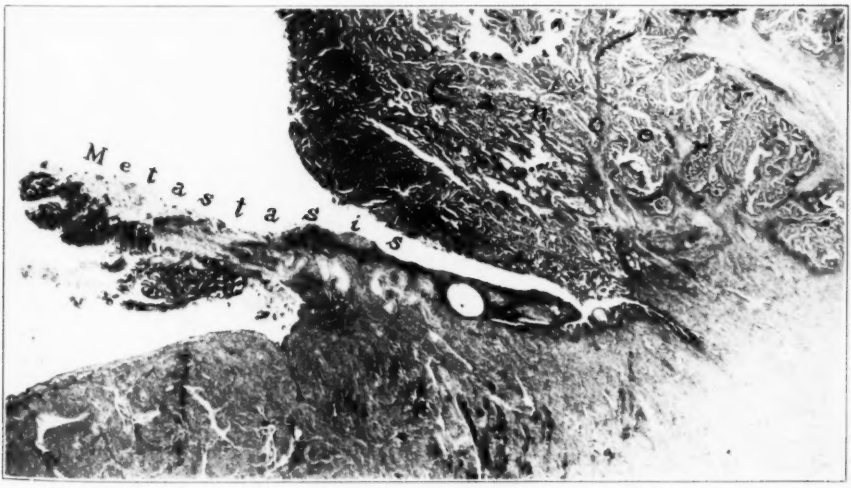

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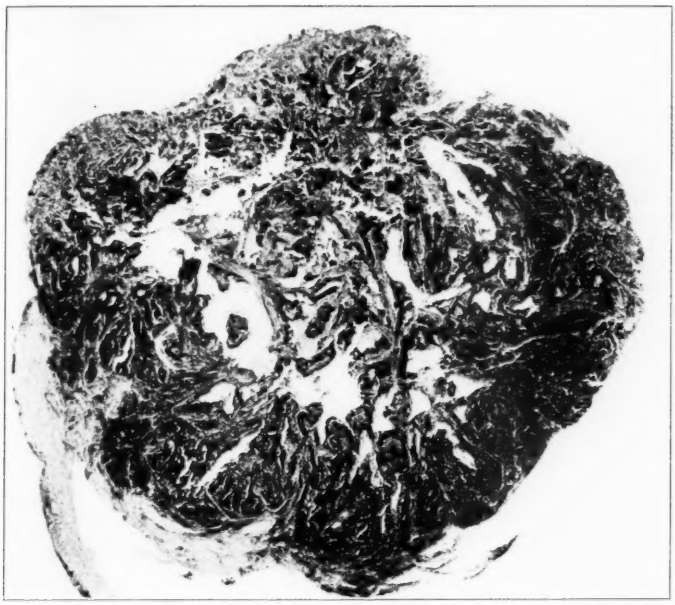
Plate 14

Fig. 38. Photomicrograph of a section of a metastatic carcinoma of the anterior vaginal wall, secondary to an advanced carcinoma of the uterus (Case 6). The carcinoma was situated in the anterior vaginal wall beneath the urethra and was partially exposed. It is impossible to state the method of its origin, whether by implantation or retrograde metastasis through the lymph vessels or veins. The patient had a diagnostic curettage a year ago and repeated X-ray treatments since then in another hospital. Compare with Fig. 113. × 10.

Fig. 39. Photomicrograph of a metastatic carcinoma of the cervical mucosa secondary to the carcinoma of the uterus (Case 6). The growth is situated on the mucosa of the posterior wall of the cervix and is not continuous with the primary tumor. The very superficial character of the growth, the absence of any lymphatic involvement and the history of a curettage a year ago all point to an implantation in a wound of the cervical mucosa. Compare with the conditions shown in Figs. 21, 36 and 37. × 10.



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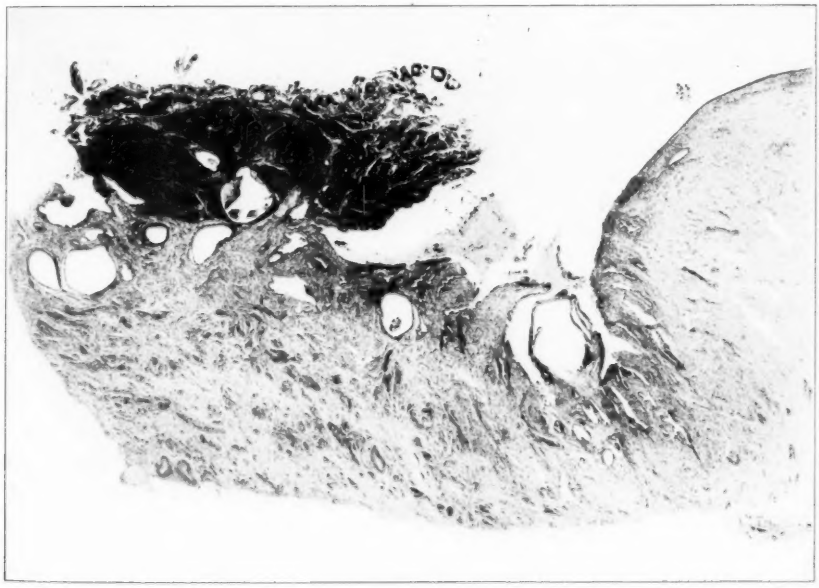

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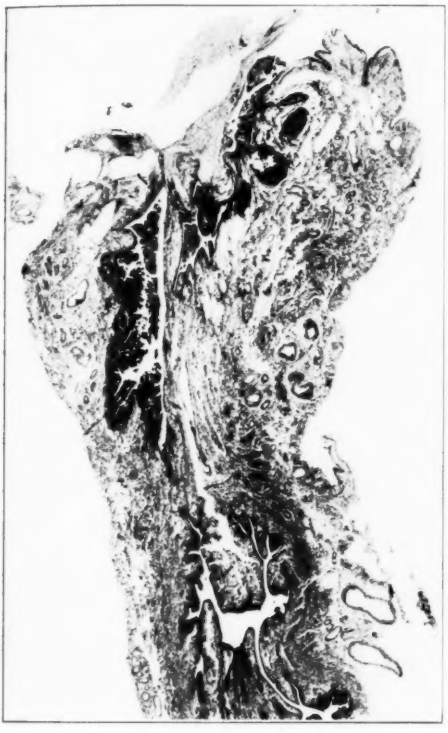
Plate 15

Fig. 40. Photomicrograph of a longitudinal section of the distal end of the left tube (Case 6). Note the superficial character of the carcinoma and the infolding of the tubal fimbriae as a result of their reaction to the tumor. In time the fimbriated end of the tube would have become occluded. Many sections of this block were studied and it was shown that what appear as multiple tumors in this photomicrograph are in reality all one growth. Is this carcinoma an instance of multicentric origin, or is it secondary to the uterine tumor by metastasis through the lymphatics or veins? Carcinoma was not found in the lymphatics of the tube. However, clumps of judged cancer cells were found in the veins of the peripheral portion of the wall of the uterus and also in one vein in the tubal wall near the uterus. Can an implantation of cancer cells escaping through the lumen of the tube be excluded? × 10.

Fig. 41. Photomicrograph (higher magnification) of a portion of the section shown in the preceding illustration. The general histological structure of the growth is that of one arising from the tubal epithelium, or of cancer cells grafted in the tubal mucosa. Other sections demonstrated that the portion of the carcinoma marked "b" was continuous with that on the surface, just as the portion marked "c" is continuous with the latter. Note the cellular débris, containing clumps of cancer cells, in the lumen of the tube. It is conceivable that some of this material might have escaped into the peritoneal cavity if it had not been prevented by the infolding and agglutination of the tubal fimbriae. It is also conceivable that it might have migrated toward the uterus. See next illustration. × 54.

Fig. 42. Photomicrograph of a portion of a cross-section of the ampulla of the tube proximal to the block from which the preceding section was made. Débris at "a" and "b," similar to that shown in the preceding illustration, is present in the lumen of the tube. A clump of cancer cells "a," similar to those marked "a" of the preceding illustration, is attached to the tubal mucosa in a cleft between two mucosal folds — a situation most favorable for splinting a graft (compare with Fig. 35). The epithelium beneath this clump has disappeared — the only area in the entire cross-section of the tube where the tubal epithelium is lacking. At this point there appears to be an intermingling of the cells of the tube with those of the clump. Many sections were made of this block and showed that clump "a" was not continuous with carcinoma elsewhere in the tube. Could the phenomenon shown here be interpreted as the grafting or implantation of cancer cells in tubal mucosa? See next illustration. × 130.

Fig. 43. Photomicrograph of a portion of the mesosalpinx of the isthmus of the tube shown in Fig. 28 demonstrating clumps of cancer cells in a lymph vessel. Many sections, some in series, demonstrated that the carcinoma in this situation was not continuous with carcinoma elsewhere in the specimen. Implantation is the only rational interpretation of the pathogenesis of this condition, which arose from the grafting of cancer cells, which had escaped into the lymphatic from the primary uterine carcinoma, on the endothelial lining of this lymph vessel. If so, could not the phenomenon in the preceding illustration well represent the grafting of cancer emboli in tubal mucosa? The fundamental stages of the development of the lesions in the two situations were the same, as is also their histological structure. × 130.



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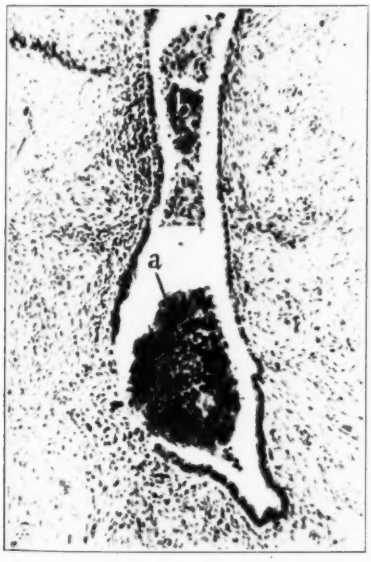

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43

Sampson

Carcinoma of Tubes and Ovaries

PLATE 16

FIG. 44. Photomicrograph of a section of the distal end of the left tube and a portion of the ovary adherent to it (Case 7). The tube was distended with blood-tinged fluid. Scattered throughout the tubal mucosa are multiple carcinomas of various sizes, thus suggesting different ages. Four of these are shown in this photomicrograph, "a," "b," "c" and "d." The largest of these is indicated by the letter "a." Other sections demonstrated that it was attached to the tubal mucosa by a slender pedicle. Carcinoma was not found in the lymphatics or veins in any portion of the specimen. I believe that the pathogenesis of these lesions admits of only two interpretations: first that of a multicentric origin for the carcinomas in the uterus and tube, all caused by the same agent or agents acting at different times; secondly, that of implantation, cancer cells escaping from the uterine growth into the lumen of the tube and becoming grafted in the tubal mucosa with subsequent grafts from this source. × 10.

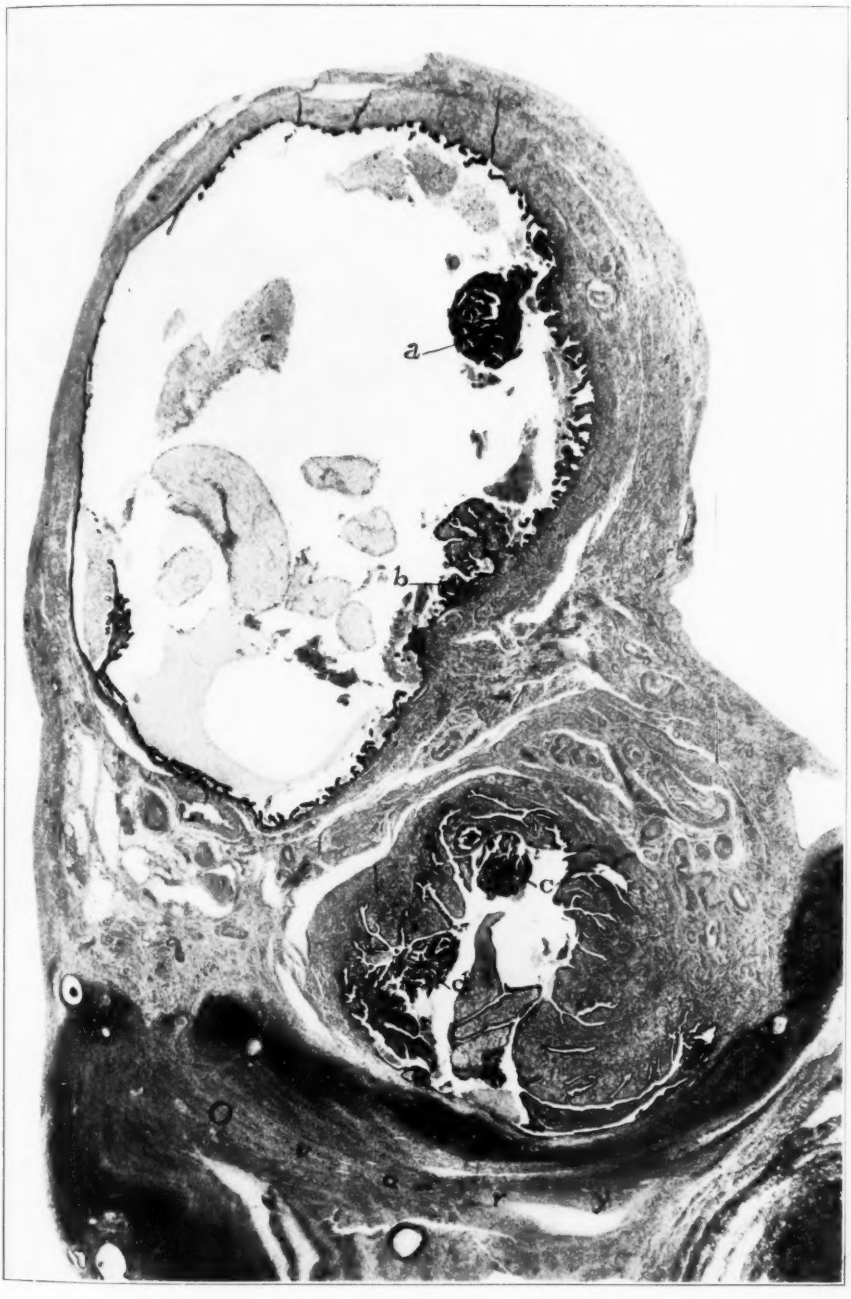
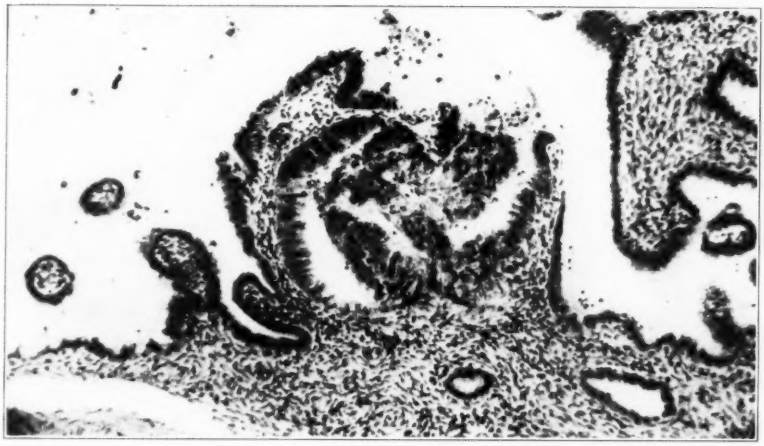

a
b
c
d

PLATE 17

FIG. 45. Photomicrograph (higher magnification) of carcinoma "b" shown in the preceding illustration. There is nothing in this section to indicate the origin of the carcinoma, whether from a differentiation of tubal epithelium, or from grafted epithelium. I believe that the relation between the malignant and the normal epithelium, as seen in the advancing edge of the growth, would be the same in either instance. The condition shown here suggests that the carcinoma might have started in a cleft between two folds of the tubal mucosa. This would be an ideal situation for the retention of a clump of cancer cells. Compare with Figs. 35 and 42. × 130.

FIG. 46. Photomicrograph of carcinoma "d" shown in Fig. 44. Note the superficial character of the growth. It has the same histological structure as that of the uterine tumor shown in the next illustration. × 25.

FIG. 47. Photomicrograph of a section of a portion of the uterine cornu including the uterine mucosa replaced by carcinoma, and the uterine portion of the tube. Unfortunately the uterine ostium of the tube was missed in trimming the block. Nevertheless, carcinoma in this situation might have extended into the uterine ostium of the tube and particles from the growing papillary outgrowths (twigs) of the carcinoma in this situation might have escaped into the lumen of the tube, just as similar fragments escape into the lumen of a lymphatic from the ends of rootlets which have penetrated these vessels. The grafting of some of the particles in the tubal mucosa could account for the secondary carcinoma of the tube. × 10.



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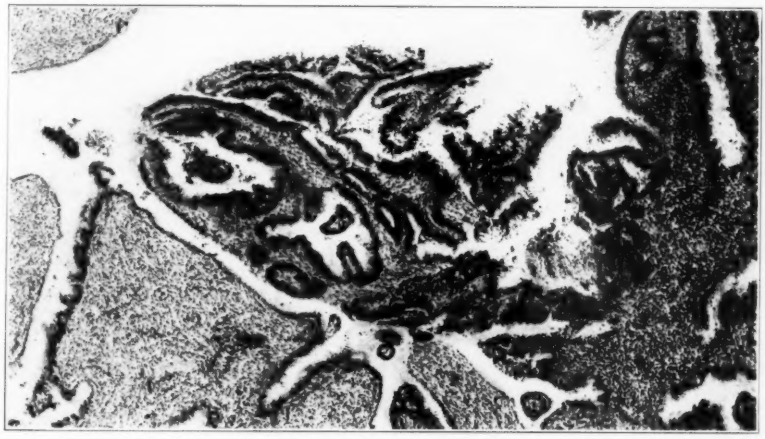

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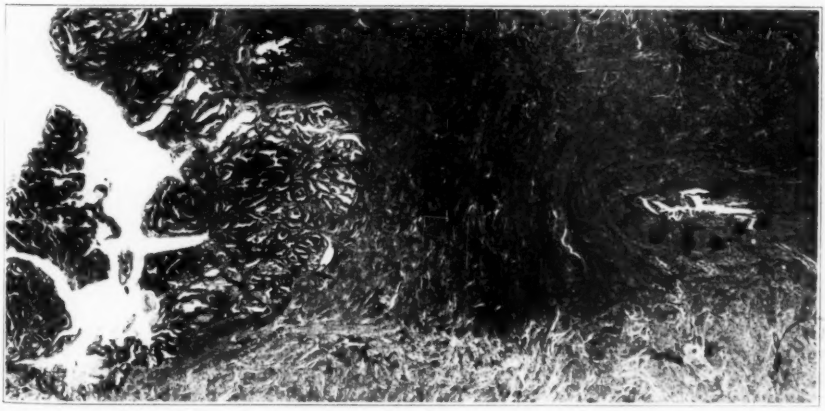

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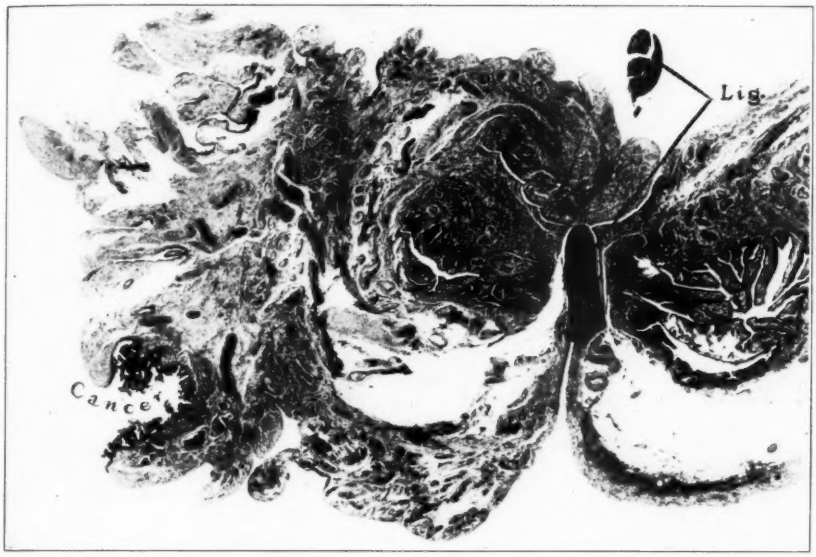
Sampson Carcinoma of Tubes and Ovaries

PLATE 18

FIG. 48. Photomicrograph of a sagittal section of the distal end of the left tube (Case 8). The uterus contained multiple myomas and an advanced adenocarcinoma of its body. Aside from peritoneal adhesions about the fimbriated end of this tube and the ovary they, as well as the opposite tube and ovary, appeared normal. There was no gross evidence of peritoneal carcinomatosis. Many sections of this block were studied and showed that the judged carcinoma of the tubal fimbriae appeared superficial in all. Carcinoma was not found in the tubal wall beneath it or in any other portion of the tube. From the histological structure of the growth as well as from circumstantial evidence, it must have arisen either from a differentiation of the tubal epithelium, or the grafting of cancer cells escaping from the uterine growth through the lumen of the tube. The latter seems more logical to me. The tube was ligated ("lig.") proximal to the fimbriated end at the operation. This would not have been done had the carcinoma of the fimbriae been recognized. × 10.

FIG. 49. Photomicrograph (higher magnification) of the carcinoma shown in the preceding illustration showing better the character of the lesion. Is it a true carcinoma or merely a hyperplasia of the tubal epithelium? See next illustration. × 54.

FIG. 50. Photomicrograph of the papillary adenocarcinoma of the uterus. There is such a marked similarity between this and the condition of the tubal fimbriae that I believe they are the same. × 54.



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Sampson

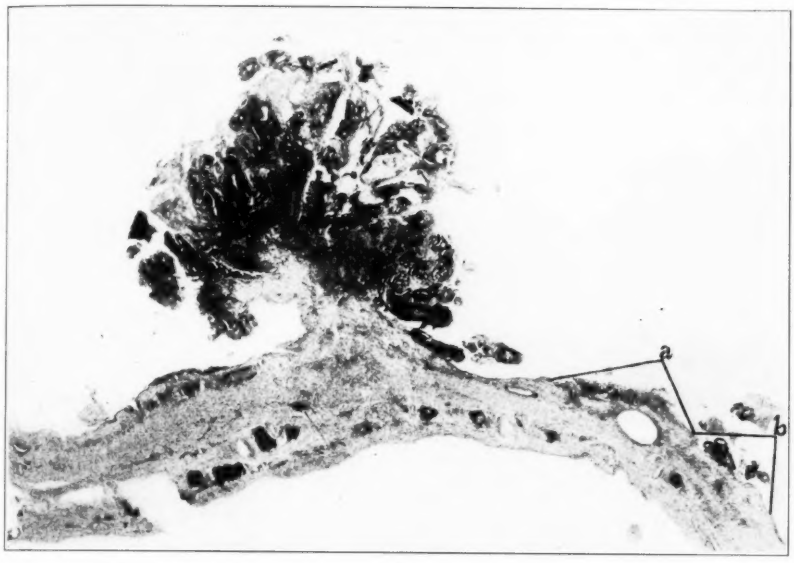
Carcinoma of Tubes and Ovaries

PLATE 19

FIG. 51. Photomicrograph of a section of the wall of a dilated tube with multiple superficial patches of adenocarcinoma in its lining. These areas are of various sizes, thus suggesting different ages. These lesions were associated with an adenocarcinoma of the body of the uterus, which had replaced the greater portion of the mucosa of the fundus, including that of both cornua (Case 9). Both tubes were dilated as in hydrosalpinx, with occlusion of their abdominal ostia and contained multiple carcinomas, as indicated in this photomicrograph. The multiple carcinomas of different sizes in the two tubes indicate either a multicentric origin, multiple metastases through the blood or lymph stream, or the implantation of particles of cancer which we realize must have been floating about in the lumen of the tube. Cancer emboli were not found in the veins or lymphatics of the tubal wall, but were present in the lumen of the tube. All the tubal carcinomas were superficial. Compare with the judged implantation carcinomas shown in Figs. 21, 36 and 39. × 10.

FIG. 52. Photomicrograph (higher magnification) of area "a" of the preceding illustration. Note the very superficial character of the growth, that it has replaced the tubal mucosa at the right and has arched over the tubal epithelium to the left to become attached again to the tubal wall. × 54.

FIG. 53. Photomicrograph (higher magnification) of area "b" of Fig. 51, emphasizing the superficial character of the growth in this situation and creating the impression that it has been added to the surface of the lining of the tube. × 54.



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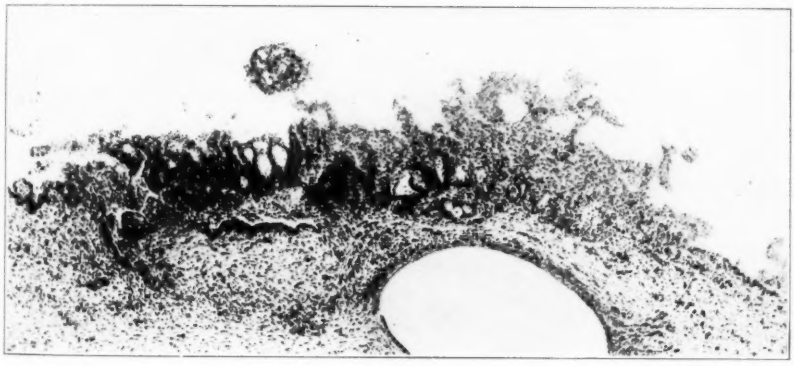

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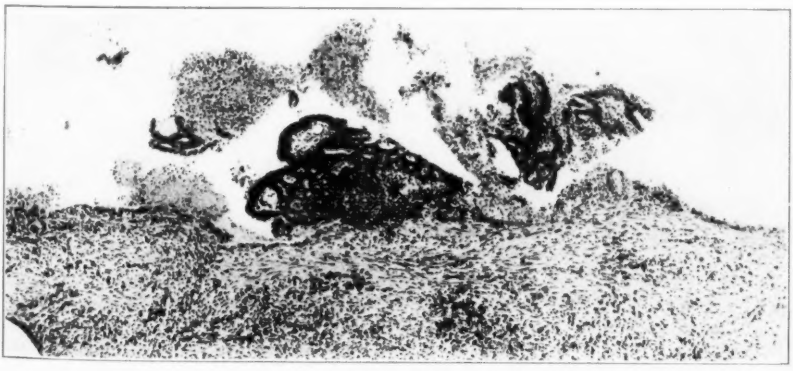

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Sampson

Carcinoma of Tubes and Ovaries

PLATE 20

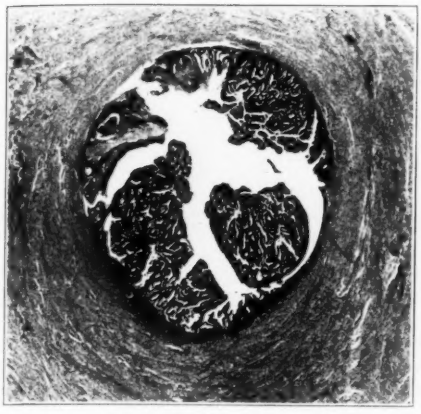
FIG. 54. Photomicrograph of one of a series of sections of the uterine cornu and uterine portion of the tube (Case 9). By continuous extension the carcinoma of the uterus has invaded the uterine portion of the tube through its uterine ostium. Note the superficial character of the growth of the same histological structure as that shown in Fig. 51, and the way it has replaced the tubal mucosa. × 10.

FIG. 55. Photomicrograph of a section of the uterine portion of the tube from the same series shown in Fig. 54, but distal to the latter. The carcinoma in this section is continuous with that shown in the preceding one and has occluded the lumen of the tube (compare with Fig. 30). It is attached to the tubal wall in only one area. It is conceivable that particles of cancer from such a growth might as readily escape into the lumen of the tube beyond it, as cancer emboli escape from a similar growth that has penetrated the lumen of a vessel. See next illustration. In both instances we are dealing with the advancing (invading and therefore growing) part of the tumor. × 10.

FIG. 56. Photomicrograph of a section of the uterine portion of the tube distal to the section shown in the preceding illustration. Note the carcinoma in the lumen of the tube. Serial sections demonstrated that some of this was continuous with the growth shown in the preceding illustration. Free particles of the growth, however, are present, which, with the occlusion of the lumen of the tube by the carcinoma shown in Fig. 55, might migrate in the tube towards its fimbriated end and even escape into the peritoneal cavity if the abdominal ostium were patent. × 54.

FIG. 57. Photomicrograph of a portion of a cross-section of the isthmus of the tube shown in the preceding illustration. The lumen of the tube is becoming occluded by an unusual reaction. See next illustration. × 54.

FIG. 58. Photomicrograph (higher magnification) of a part of the section shown in the preceding illustration. I do not know how to interpret the condition shown here. I am tempted to believe that it must be a reaction arising from something escaping into the lumen of the tube from the growth in the uterus, or that in the tube. Are the large epithelial cells, to the left, atypical cancer cells or hypertrophied and distorted tubal epithelium? What is the relation between the carcinoma of the uterus and that in the two tubes? Are all the isolated carcinomas in the three situations tumors of multicentric origin due to the same agent, or agents acting at different times? The escape of cancer cells from the uterine growth into the lumina of the tubes, with subsequent implantation on the lining of the tubes followed by secondary implantations, would account for the condition present in these three situations. × 130.



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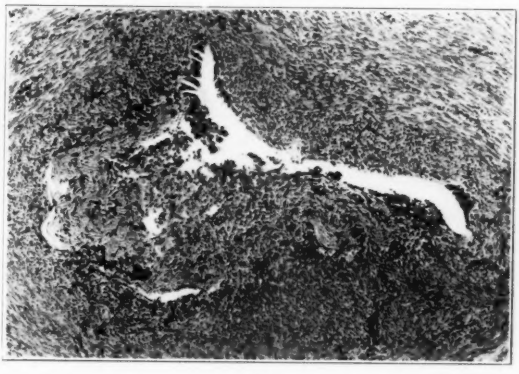

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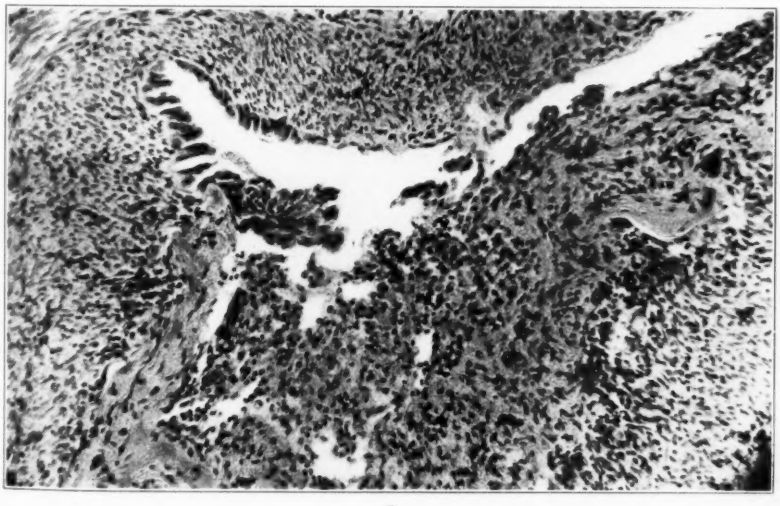


58

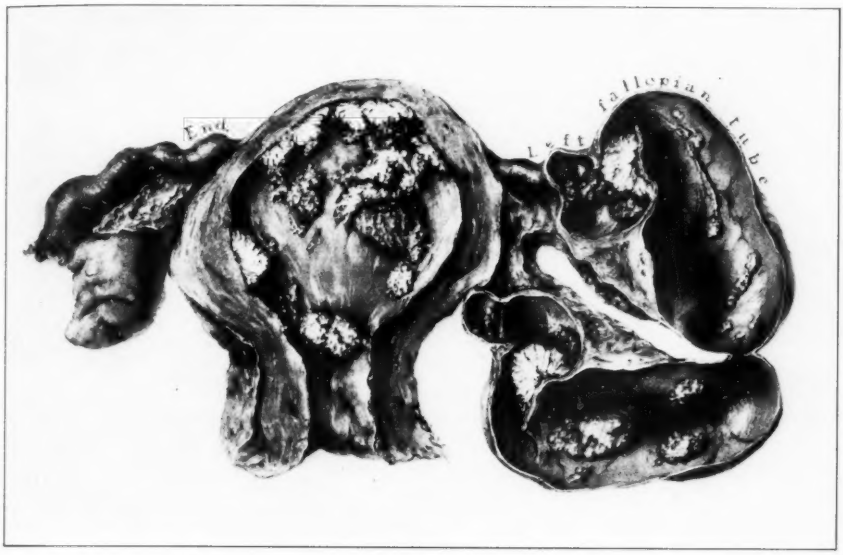
Sampson

Carcinoma of Tubes and Ovaries

PLATE 21

FIG. 59. Papillary adenocarcinoma of the body of the uterus and of the left tube (Case 10). Multiple carcinomas of various sizes, therefore suggesting different ages, are present in both the uterus and the tube. Is this an example of the multicentric origin of carcinoma due to the same agent or agencies acting at different times, causing a like differentiation of uterine and tubal epithelium into carcinoma? Is it possible that the growth in both the uterus and tube may have spread by the implantation of cancer cells escaping into these cavities? What is the relation between the carcinoma of the uterus and that of the tube? Could one be secondary to the other? Why is carcinoma present in the left tube and not in the right? See Figs. 61 to 68, inclusive. × 2/3.

FIG. 60. Photomicrograph of a section of the largest growth present in the left tube. Similar and even larger growths were present in the uterus. Note the superficial character of the tumor. Many sections (some in series) were made of this block and it was demonstrated that the carcinoma had spread from the base of this papilloma, replacing the tubal epithelium. On inspecting the surface of this papilloma, one can readily understand how clumps of living cancer cells might break off from the growing tips of the papillary outgrowths and be disseminated into the cavity of the tube, just as cells escape into the peritoneal cavity from a similar growth on the surface of the ovary or as carcinoma escapes into the lumen of a lymph vessel. The small isolated growths in the tube are not unlike implantations on the peritoneum and in lymph vessels. × 10.



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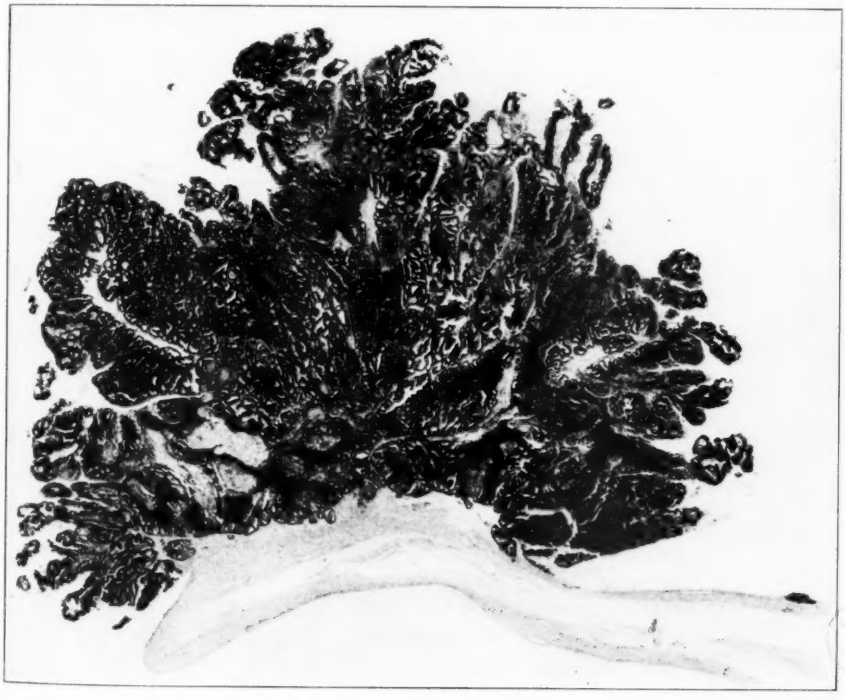

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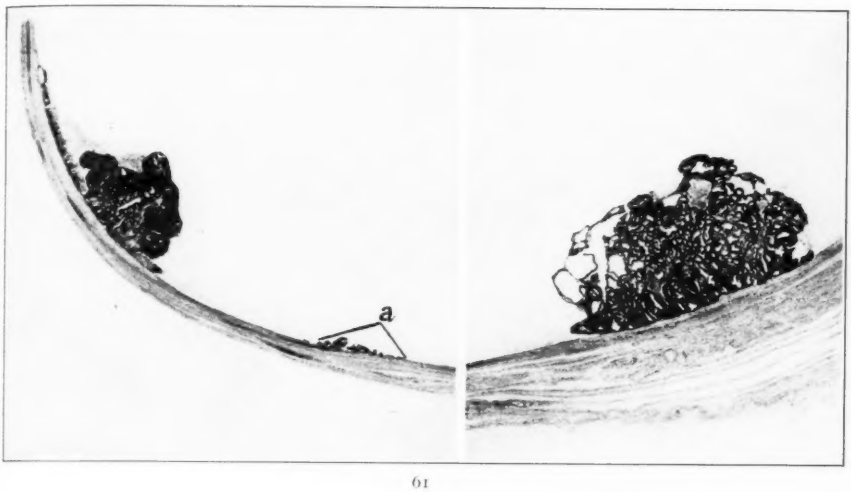
Sampson Carcinoma of Tubes and Ovaries

PLATE 22

FIG. 61. Photomicrographs of two sections of the tubal wall showing three discrete patches of carcinoma replacing the tubal mucosa. The variation in their sizes suggests different ages. Note that the one to the left is spreading over and replacing the tubal mucosa. Compare these with the tumors shown in the preceding illustration, all the same magnification. × 10.

FIG. 62. Photomicrograph (higher magnification) of the patch of cancer "a" of the preceding illustration. I believe that the cancer began in the center of the patch where the growth is the most advanced and probably spread in all directions. × 54.

FIG. 63. Photomicrograph (still higher magnification) of the advancing edge of the patch of carcinoma shown in the preceding illustrations. One can see that the growth has replaced the tubal epithelium and is advancing over it to the right. × 130.



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Sampson Carcinoma of Tubes and Ovaries

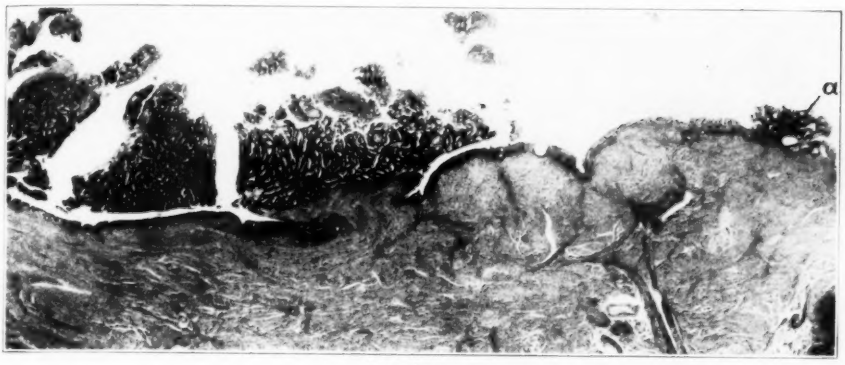
PLATE 23

FIG. 64. Photomicrograph of a portion of the uterus showing some of the smaller patches of carcinoma indicated in Fig. 59. Note the very superficial character of the growth and the way it has apparently spread over the mucosa by direct extension and also, I believe, by implantation. × 10.

FIG. 65. Photomicrograph (higher magnification) of patch "a" of the preceding illustration. Many sections of this block demonstrated that this patch of carcinoma was not continuous with that elsewhere in the specimen. Note its very superficial character, as though added to the surface of the uterine mucosa. Its pathogenesis cannot definitely be determined. However, one is impressed that in some way it must be derived from the carcinoma of the uterus near it. It is not continuous with the latter. Compare with Figs. 21, 36, 39 and 51. × 54.

FIG. 66. Photomicrograph of a section of the left cornu of the uterus, including the uterine ostium of the tube. By serial sections one could follow the carcinoma, replacing the uterine mucosa, into the ostium of the tube, thus plugging the latter, as in Figs. 30 and 55. × 10.

FIG. 67. Photomicrograph of a section of the uterine ostium of the tube, distal to the preceding section, showing carcinoma in its lumen continuous with that shown in the preceding illustration. Clumps of cancer cells from such an area might easily escape into the patent tube or even into the peritoneal cavity, should the fimbriated end be open. A study of this specimen indicates that implantation of carcinoma might have occurred in both the uterine and tubal mucosae. The last two illustrations indicate a way that cancer cells from the uterine tumor might have escaped into the lumen of the tube. The isthmus of the tube was patent. × 25.



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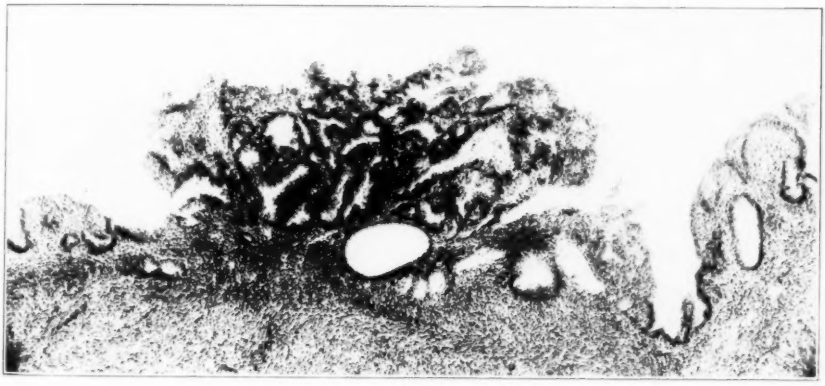

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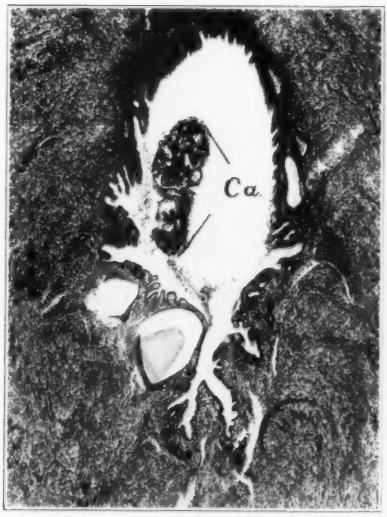


67

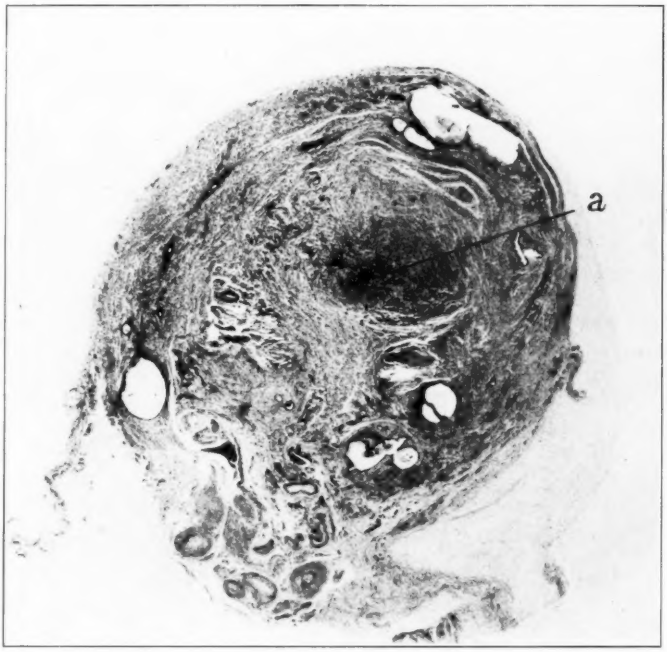
Sampson

Carcinoma of Tubes and Ovaries

PLATE 24

FIG. 68. Photomicrograph of a cross-section of the isthmus of the right tube. Carcinoma was not found in the right tube. An endosalpingeosis (see "End." of Fig. 59) is present in this tube with occlusion "a" of its lumen. This probably was present before the growth developed in the uterine mucosa, and would prevent the escape of particles of the uterine growth into the lumen of this tube, as could have occurred in the opposite tube. × 10.

FIG. 69. Photomicrograph of a section of the cervix (Case 10), showing early squamous cell carcinoma "c" arising from the mucosa of the vaginal portion of the cervix near its junction with the mucosa of the cervical canal. This is the strongest argument in the case favoring the multicentric theory for the origin of the many carcinomas of different sizes in the uterus and left tube. Even so it does not prove that these carcinomas are not of implantation origin. × 10.



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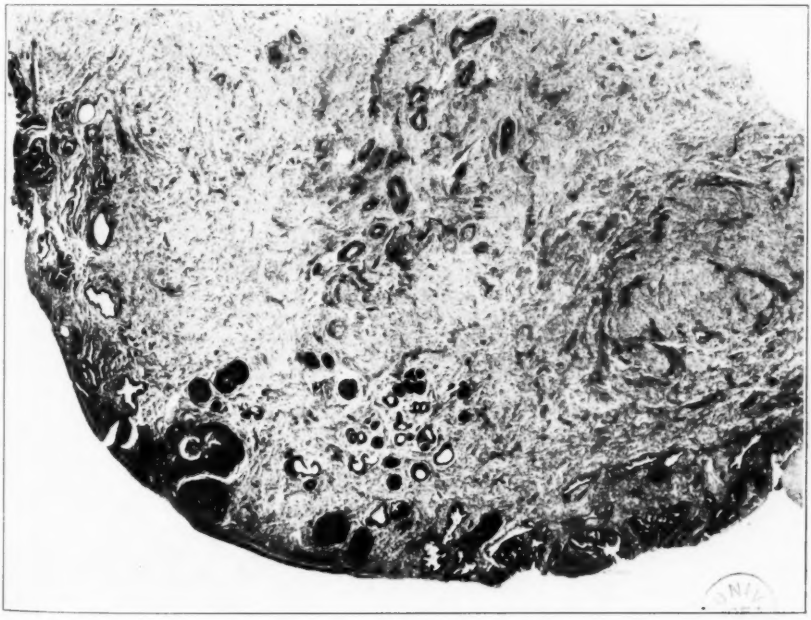

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Sampson Carcinoma of Tubes and Ovaries

PLATE 25

FIG. 70. Adenocarcinoma arising in the right cornu of a markedly bicornuate uterus (Case 11). Both tubes and ovaries had been removed with the uterus. Carcinoma was not found in either the left tube or ovary but was present in the right tube and ovary. Natural size.

FIG. 71. Photomicrograph of a section of the advancing margin of the cancer. Note the superficial character of the growth and the way it replaces and rides over the adjacent uterine mucosa. × 54.

FIG. 72. Photomicrograph of a section of the uterine ostium of the right tube showing clumps of cancer cells in its lumen undoubtedly derived from that portion of the growth filling the right uterine cornu (see Fig. 70). Similar clumps of cells might easily have gone farther into the lumen of the tube and even out through the fimbriated ostium into the peritoneal cavity. × 54.

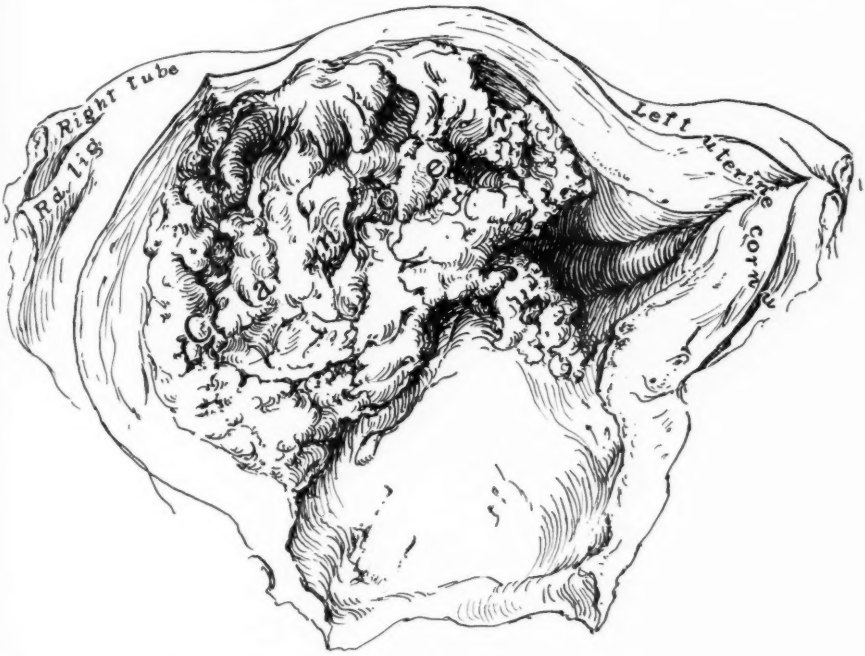

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PLATE 26

FIG. 73. Photomicrograph of a section of the right ovary and portion of the distal end of the occluded tube fused with the surface of the ovary, as may occur in hydrosalpinx (Case 11). Cancer "c" is present in the newly formed tissue which has developed between the ovary and wall of the tube. The small cyst of the ovary is lined by carcinoma. Compare with Fig. 108. × 5.

FIG. 74. Photomicrograph of a section of a portion of the ovary and the occluded fimbriated end of the tube which is fused with the ovary. Carcinoma developing in the fimbriae of the tube might have caused an infolding of these structures and occlusion of the tube, as occurs in inflammatory conditions and also in other instances of carcinoma in this situation (see Fig. 40). The subsequent growth of the carcinoma in this situation might have invaded the ovary and given rise to the conditions indicated in this and the preceding illustration. What was the pathogenesis of the carcinoma of the tubal fimbriae? I believe that it could have arisen from the transtubal migration of particles of cancer escaping from the primary uterine growth. Others may claim that it reached the fimbriae through the lymph or blood streams. See next illustration. × 10.

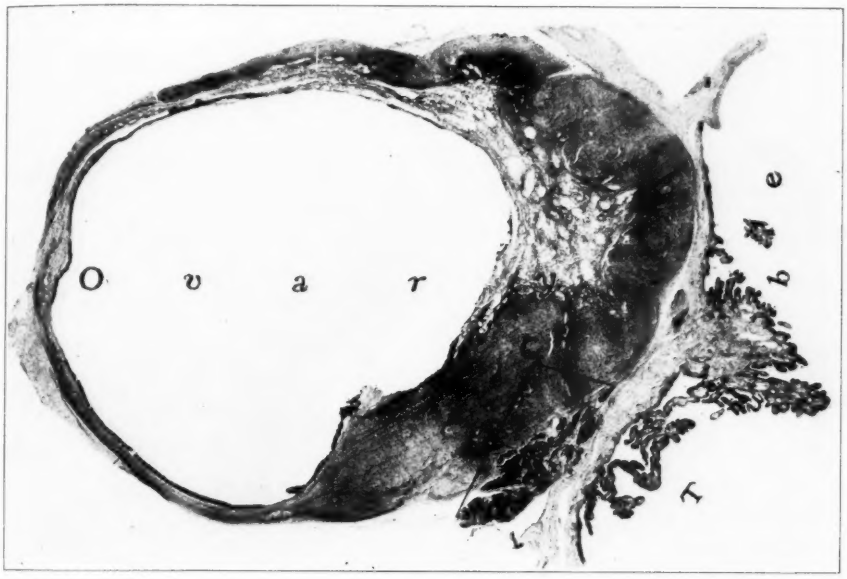

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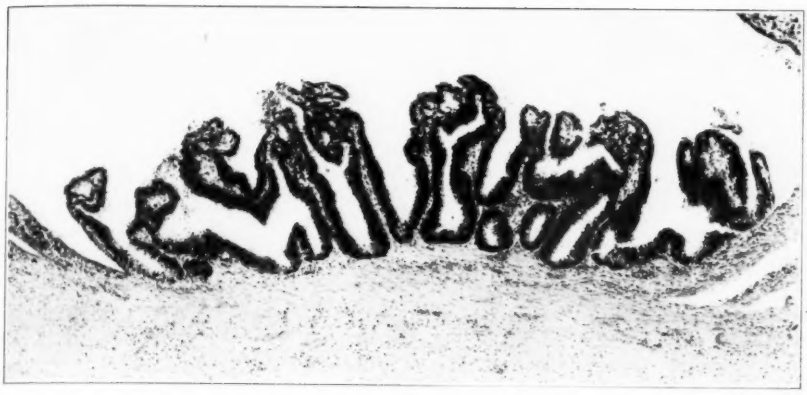
Plate 27

Fig. 75. Photomicrograph of a relatively early patch of carcinoma of the mucosa of the distal portion of the ampulla of the tube. Note the relation of the advancing cancer cells to the normal tubal epithelium on both sides of this patch. The carcinoma probably developed near the center of the patch. I believe that carcinoma starting either as a graft or from a differentiation of tubal epithelium (multicentric origin) could have given rise to the histological picture shown in the sections of this patch of carcinoma. Metastasis through the lymph or blood stream could not have given rise to the condition shown in this or the next photomicrograph. × 54.

Fig. 76. Photomicrograph of apparently an older patch of carcinoma which has developed in the tubal mucosa. This patch of carcinoma, as the preceding one, was isolated and not continuous with that elsewhere in the specimen. Note the very superficial character of the growth, as though added to the surface of the tubal mucosa. Compare with Figs. 21, 36, 39, 51 and 65. × 25.

Fig. 77. Photomicrograph (higher magnification) of the section of the tubal wall shown in the preceding photomicrograph, to the right of the patch of carcinoma. The tubal epithelium is lacking in the center of the photomicrograph. Through this break newly formed tissue has poured into the lumen of the tube. Enmeshed in this newly formed tissue are clumps of epithelial cells. It is impossible to state whether they are cancer cells or clumps of hyperplastic desquamated tubal epithelium. Naturally the question arises as to what could have caused this reaction, other than the carcinoma in the tube or something derived from it. This area might provide ideal soil for the grafting of cancer cells floating about in the lumen of the tube. × 130.



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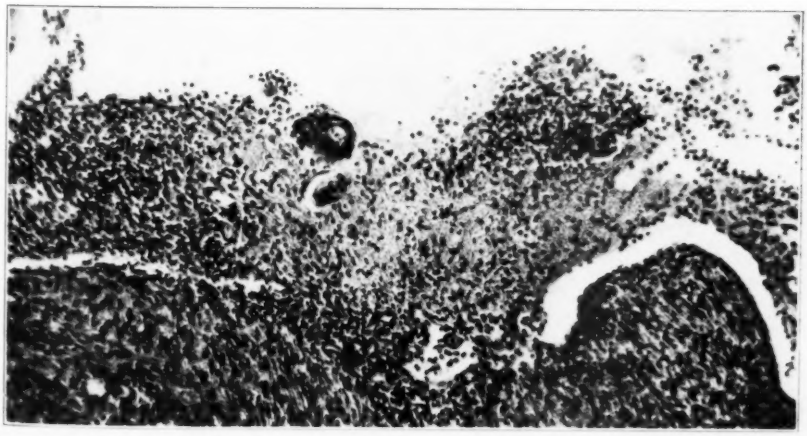


77

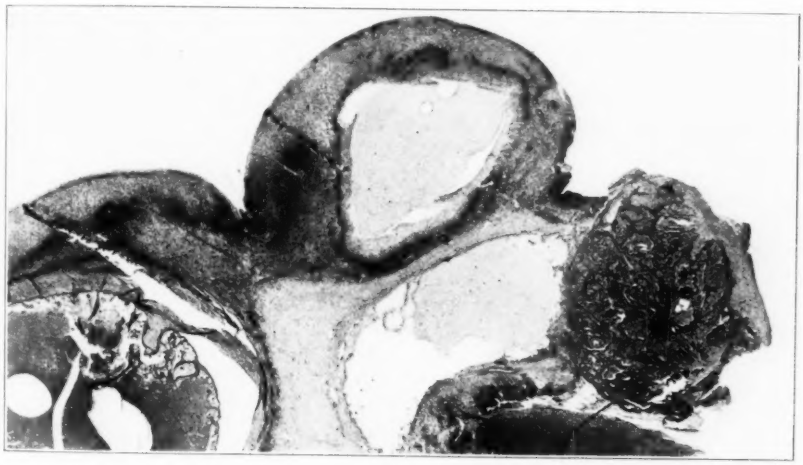
Sampson Carcinoma of Tubes and Ovaries

Plate 28

Figs. 78 and 79. Photomicrographs of sections of two metastatic carcinomas of the left ovary, associated with an adenocarcinoma of the body of the uterus (Case 12). The bottom of the posterior cul-de-sac was obliterated by a similar growth fusing the posterior wall of the cervix with the sigmoid and presenting in the posterior vaginal vault, as frequently occurs in endometriosis in this situation. Carcinoma in tissue spaces and possibly lymphatics of the ovary was found only about the periphery of the two metastatic tumors, and was judged to represent invasions by the metastatic tumors and not necessarily indicate their pathogenesis. The superficial character of the ovarian metastases, as well as the obliteration of the bottom of the cul-de-sac by the carcinoma, strongly favors the dissemination of carcinoma through the patent tubes with implantation on the surface of the ovary and peritoneum of the cul-de-sac. Exact duplication of the conditions found in this case are frequently encountered in endometriosis. × 10.

Fig. 80. Photomicrograph (higher magnification) of area "a" indicated in preceding photomicrograph. The character of the metastatic tumor, identical with that present in the uterus, is shown at the right. To the left are cells "c" on the surface of the ovary similar to those of the metastatic tumor, and associated with a definite reaction of the underlying ovarian tissue similar to that caused by an irritant applied to the surface of the ovary. It well might represent the implantation of cancer cells in this situation. × 54.

78



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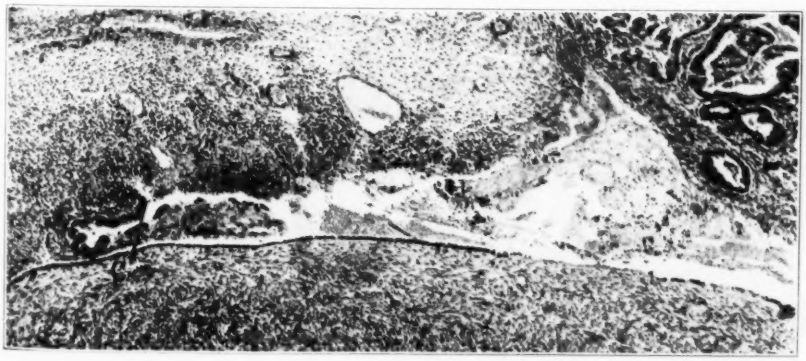
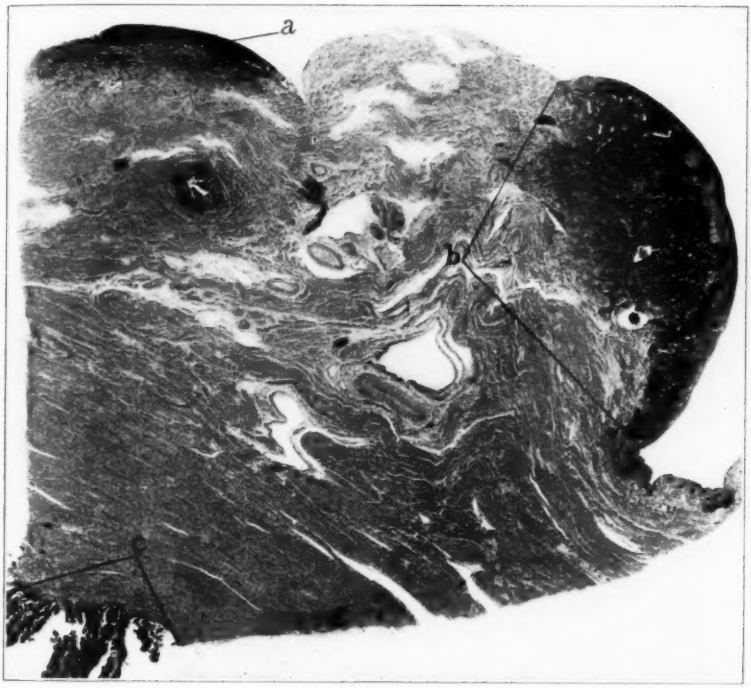


80

PLATE 29

FIG. 81. Photomicrograph of a section of a portion of the right uterine cornu including the tube and a small part of the uterine mucosa (Case 13). The patient had a large endometrial tumor distending the uterine cavity and protruding through the cervical canal. This was judged to be a stromal cell sarcoma, or possibly an atypical carcinoma. A papillary adenocarcinoma was also present in other portions of the uterine mucosa. There was a general peritoneal sarcomatosis, including the involvement of the omentum. The normal appearing tube is shown in cross-section with clumps of cells lying free in its lumen which might be malignant. At "c" is a small portion of the uterine mucosa replaced by a papillary adenocarcinoma. At "a" and "b" are two subperitoneal or peritoneal metastases, which appear to be invading the peritoneum from within. Clumps of cancer cells are present in lymphatics beneath the base of the larger tumor. This does not prove the pathogenesis of the metastasis since an implant might have the same potentialities of lymphatic invasion as a primary tumor. While implantation metastases cannot be excluded the whole picture suggests to me metastases through the lymph channels. Compare with "a" of Fig. 1 and "s" of Fig. 7. × 10.

FIG. 82. Photomicrograph (higher magnification) of a portion of the larger metastasis shown in the preceding illustration. Note the malignant tumor in the lymphatic "L," but more important the apparent growth of the tumor towards the peritoneum from within. Compare with Figs. 3 and 11, and also with Figs. 84, 85 and 87. × 54.



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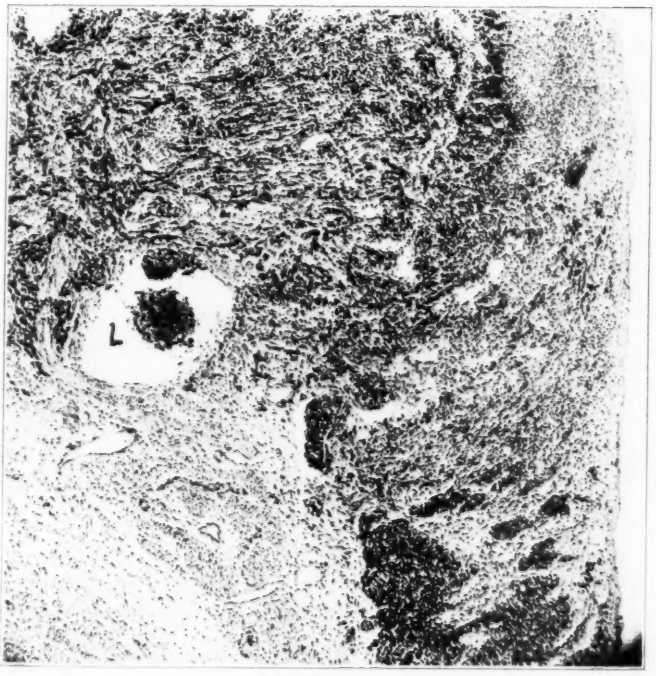


82

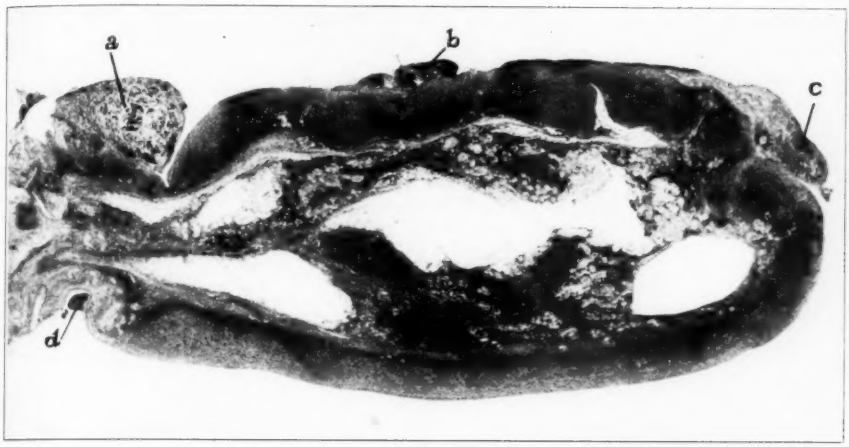
Sampson Carcinoma of Tubes and Ovaries

PLATE 30

FIG. 83. Photomicrograph of a section of the ovary (Case 13). Metastases "a," "b," "c" and "d" are present on the surface of the ovary without involvement of the lymph vessels of that organ. The ovarian tissue, beneath the base of the largest metastasis "a," has been invaded by the tumor. In all the other three metastases the entire tumor is in newly formed tissue on the surface of the ovary. Similar metastases were present on the surface of the opposite ovary and were all a part of a general peritoneal sarcomatosis, all of which had the same histological structure as that of the uterine tumor. These metastases differ in their histological structure from those of the uterine cornu shown in Fig. 81. These are supraperitoneal and the others are subperitoneal. × 8.

FIG. 84. Photomicrograph (higher magnification) of metastasis "d" of the preceding photomicrograph. Note the very superficial character of the growth, without any evidence of the tumor in the deeper tissues of the ovary. The tumor cells must have been added to the surface of the ovary or have arisen from a differentiation of its surface epithelium, *i.e.*, of multicentric origin. × 54.

FIG. 85. Photomicrograph (higher magnification) of metastasis "b" indicated in Fig. 83. The entire tumor is situated in newly formed tissue which has developed on the surface of the ovary. The tumor cells are encapsulated in this tissue as foreign bodies become encapsulated on the surface of the peritoneum. Compare with Figs. 16 and 17 where a somewhat similar lesion has resulted from the direct extension of carcinoma through the uterine wall. × 25.



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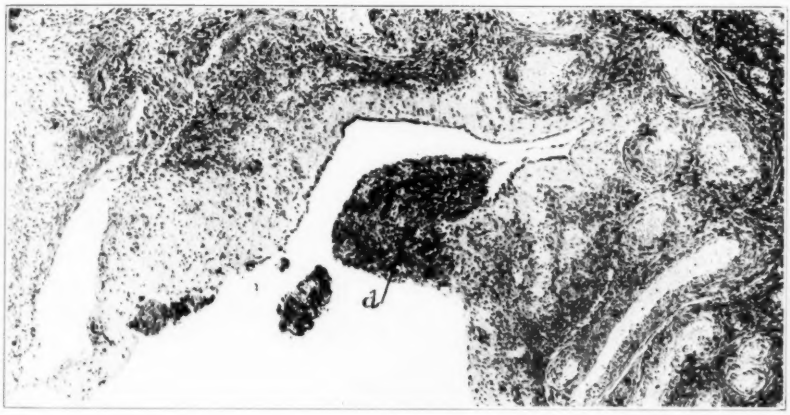

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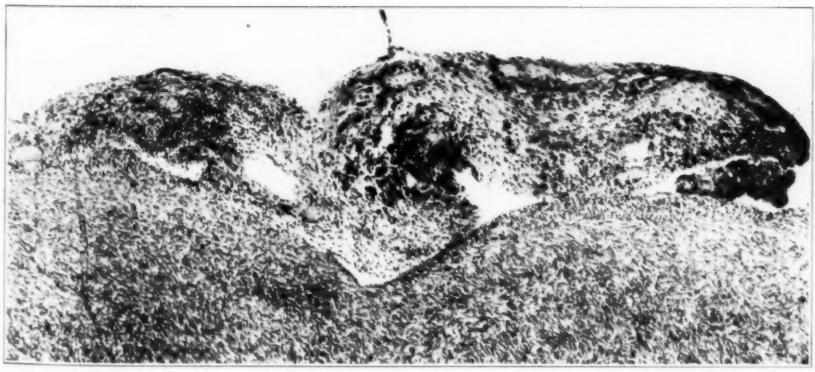

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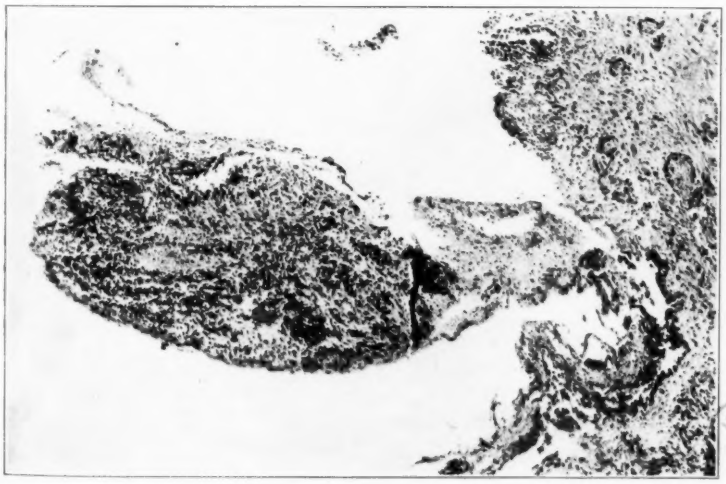
PLATE 31

FIG. 86. Photomicrograph of a cross-section of the ampulla of the tube demonstrating metastases to its peritoneum, similar to those found on the ovaries and elsewhere throughout the peritoneal cavity. Metastasis "a" is very superficial with only a slight invasion of the underlying peritoneum, which is only slightly thickened. In metastases "b" and "c" the peritoneum is markedly infiltrated with carcinoma and therefore greatly thickened, and might be confused with metastases of lymphatic origin. Metastasis "d" has developed in newly formed tissue and is attached by a slender pedicle of the same to the peritoneum of the mesosalpinx. Carcinoma was not found in the lymphatics of the mesosalpinx or of the tubal wall. × 8.

FIG. 87. Photomicrograph (higher magnification) of the metastasis "d." I believe that the pathogenesis of this lesion is the same as that of similar implantation metastases found in peritoneal carcinomatosis of ovarian origin and well shown in Figs. 36 and 37 of a previous paper.[9] × 54.

86



87

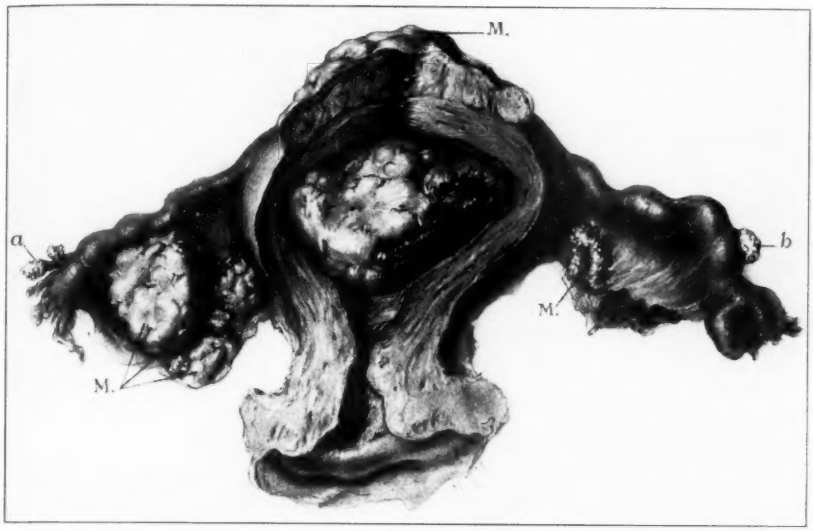
Sampson Carcinoma of Tubes and Ovaries

PLATE 32

FIG. 88. Uterus and tubes (ovaries are not visible as they lie behind the tubes and broad ligament) of an advanced adenocarcinoma of the body of the uterus, associated with an extensive peritoneal carcinomatosis including the omentum and diaphragm, as in extensive peritoneal carcinomatosis of ovarian origin (Case 14). Peritoneal metastases "M," "M" and "M" are present on the surfaces of the uterus and both broad ligaments. At "a" is a metastasis of the fimbriae of the right tube and "b" a metastasis to the peritoneum of the left tube. The judged primary tumor arose from the mucosa of the posterior wall of the uterus and has distended the uterine cavity and spread into both uterine cornua. × 2/3.

FIG. 89. Photomicrograph of the uterine mucosa at one side of the main tumor, a typical papillary adenocarcinoma. Since this extended into both uterine cornua particles of the carcinoma could have gained access easily to the uterine ostia of the tubes. × 54.

FIG. 90. Photomicrograph of a section of the uterine wall beneath the main tumor. It has been almost entirely replaced by the growth, which is partly necrotic. × 54.



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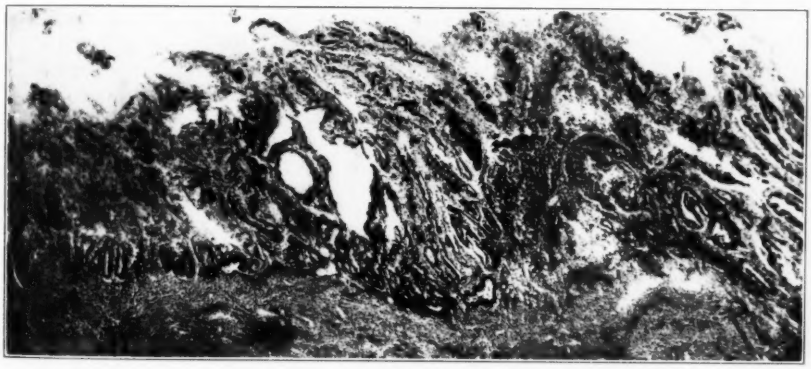

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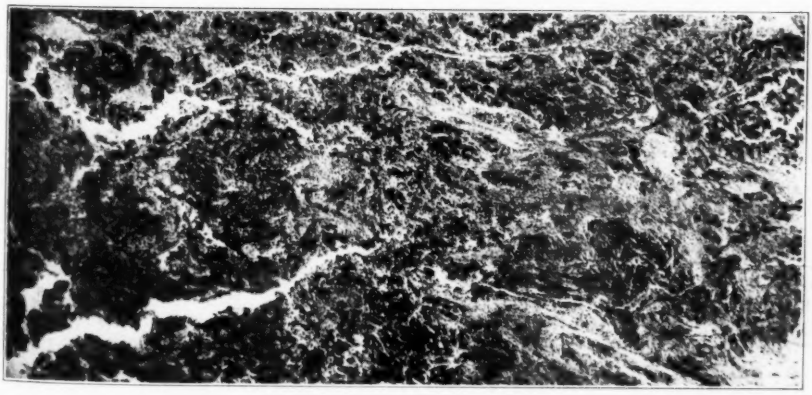

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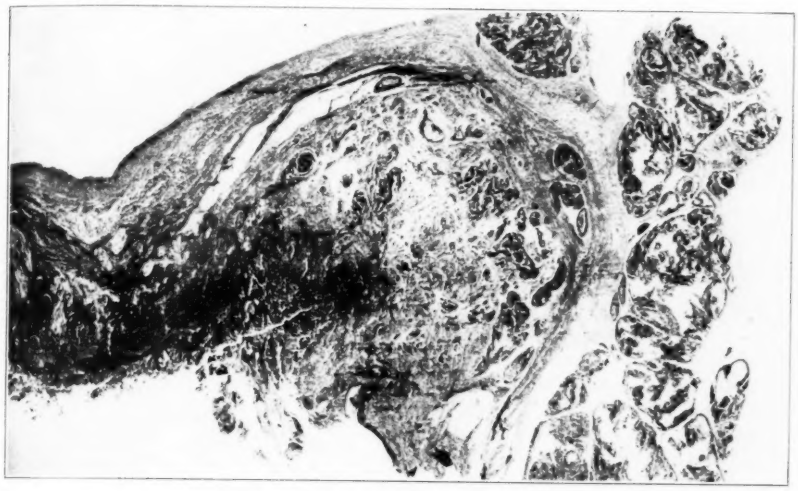
PLATE 33

FIG. 91. Photomicrograph of a section of a portion of the uterus beneath the tumor shown in Fig. 88. Since the main tumor was necrotic and very friable the greater portion of it became detached from the uterine wall in cutting the blocks and therefore does not appear in this section. Note the extensive infiltration of the uterine wall by the carcinoma and the encapsulated growth on the peritoneal surface of the uterus to the right. Although many sections from this block were studied I was unable to demonstrate a continuity between the growth in the uterine wall and that on its peritoneal surface. I believe that the latter was of metastatic origin, as is the metastasis shown in the next illustration. Both were a part of an extensive peritoneal carcinomatosis. × 3.

FIG. 92. Photomicrograph of a section through the right ovary, the tube, and the large metastasis of the anterior layer of the right broad ligament (see "M" of Fig. 88). Carcinoma was not found in any of the lymphatics. Therefore, lymphatic permeation can be excluded. In this section carcinoma is present only in the metastasis of the peritoneum of the anterior layer of the broad ligament and on the surface of the ovary at "a" and "b" (see also Figs. 94 and 95). The histological structure of the metastasis on the surface of the broad ligament and that of the peritoneum of the uterus, shown in the preceding illustration, is the same and I believe that both of them had a like origin, beginning in the same way as the metastasis shown in Fig. 101. × 3.

FIG. 93. Photomicrograph of a section of the distal end of the right tube including the carcinoma of the tubal fimbriae shown in "a" of Fig. 88. Did the carcinoma of the fimbriae arise from a differentiation of tubal epithelium or from the implantation of cancer cells on the surface of the fimbriae? I believe the latter, but realize that it cannot be proved. It is a non-encapsulated implant, *i.e.*, of the grafted and not the foreign body type. Compare with Figs. 96, 99 and 100. × 10.



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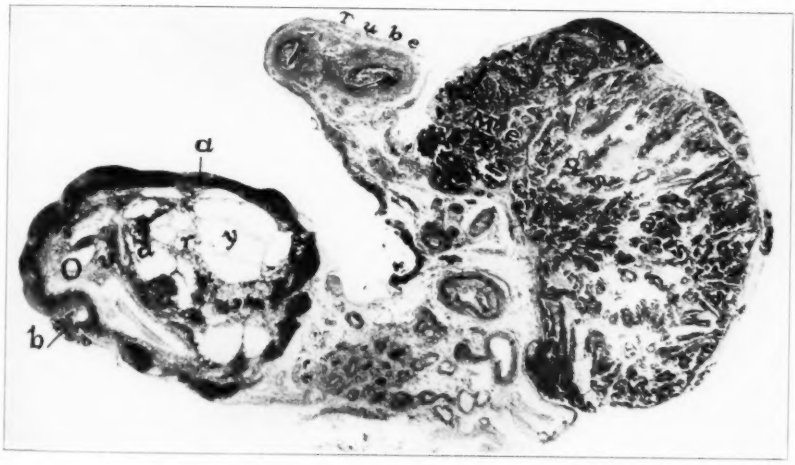

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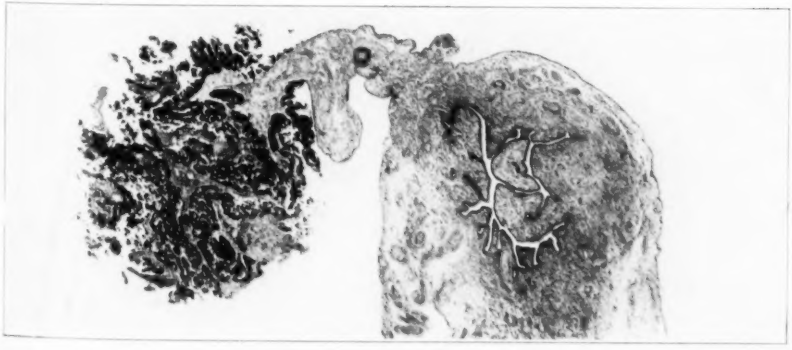


93

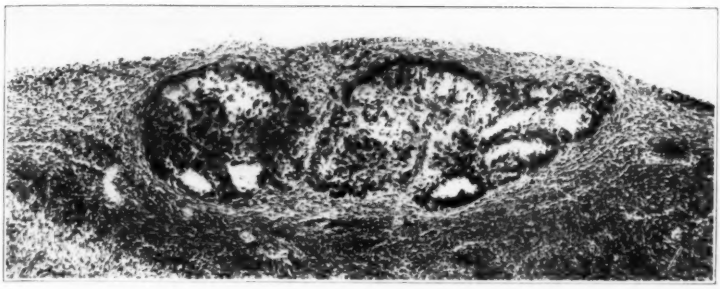
Plate 34

Fig. 94. Photomicrograph (higher magnification) of the metastasis to the right ovary indicated by "a" of Fig. 92. It is an encapsulated metastasis and might be assumed to be of lymphatic origin. Carcinoma, however, was not found in the lymphatics of the ovary. The encapsulation is identical with that which follows the escape of foreign bodies into the peritoneal cavity, thus suggesting that this metastasis is of implantation origin. See Fig. 2 of previous paper,[9] also compare with Fig. 96 of present paper. × 54.

Fig. 95. Photomicrograph (higher magnification) of the metastasis of the right ovary indicated by "b" of Fig. 92. It is a partially encapsulated metastasis and could be also of implantation origin. See next illustration. × 25.

Fig. 96. Photomicrograph of a section of a portion of the wall of the sigmoid and its mesentery (Case 14). The two metastases have the same histological structure as those of the ovary shown in the two preceding illustrations and, I believe, had a like origin. × 25.

Fig. 97. Photomicrograph of a section of a portion of the wall and lumen of the right tube showing clumps of cancer cells "C" lying free in the lumen. It is impossible to state whether they entered the tube through its abdominal or uterine ostium. Carcinoma of the mucosa was not present in this tube. Whatever their origin, it demonstrates the transtubal migration of cancer cells. Unfortunately, sections were not made of the uterine ostia of the tubes, but the growth occupied both uterine cornua. × 54.



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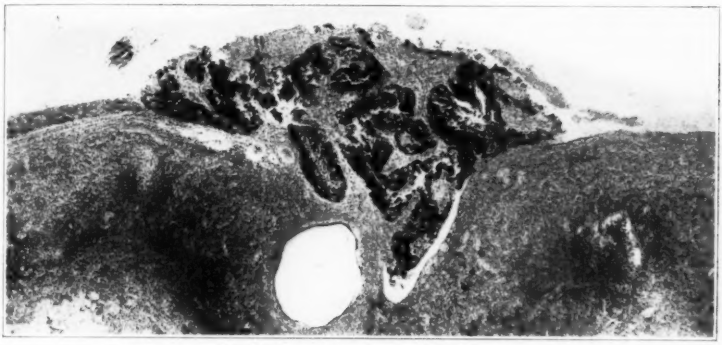

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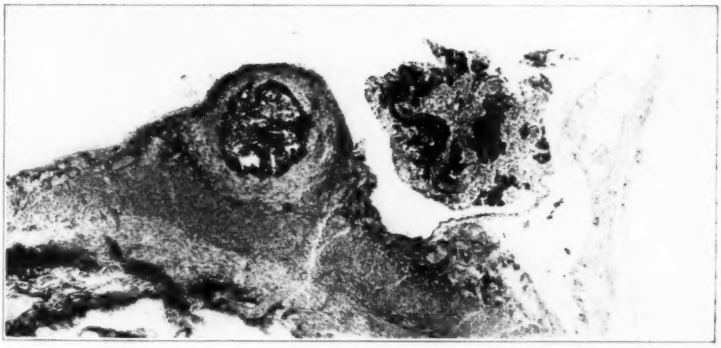

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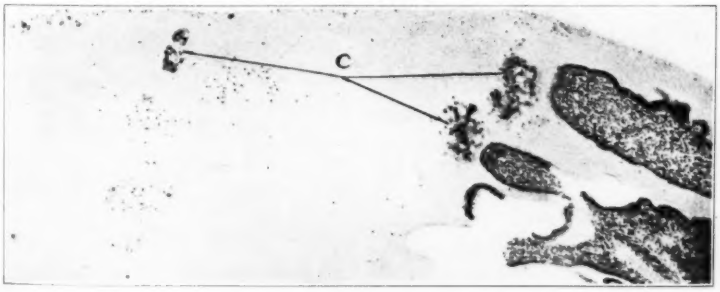
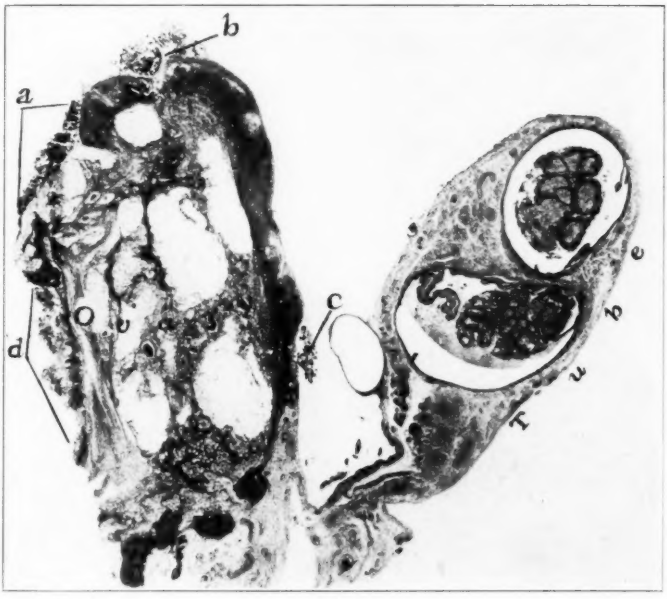


97

PLATE 35

FIG. 98. Photomicrograph of a cross-section of the left ovary and tube shown in Fig. 88. Metastatic carcinoma is present on the surface of the ovary as indicated by "a," "b," "c" and "d." For a higher magnification of implants "a" and "c" see Figs. 103 and 104. These implants are very superficial and without invasion of the deeper ovarian tissue. Two of the three secondary carcinomas of the tube are shown. All are of about the same size and attached to the tubal mucosa by slender pedicles (see Fig. 100). Carcinoma was not found in any of the lymph vessels of the ovary, tube or mesosalpinx. × 5.

FIGS. 99 and 100. Photomicrographs of two cross-sections of the tube from a series of sections taken through metastasis "b" of Fig. 88. The peritoneal metastasis is attached to the surface of the peritoneum by a pedicle, as shown in Fig. 99. The tubal carcinoma is attached to the tubal mucosa by a pedicle "p" of Fig. 100. Carcinoma was not found in the lymphatics of the tube. Although the peritoneal metastasis is encapsulated the growth has penetrated the capsule in various places. The tubal tumor is non-encapsulated and is approximately of the same size as that on the peritoneum. It would seem that they probably had a simultaneous and like origin. The peritoneal tumor has the histological structure of an implantation tumor of foreign body type and that of the tube an implantation tumor of grafted type (see Figs. 101 and 102). The only other rational explanation for the pathogenesis of the two tumors is that they are of multicentric origin. × 10.



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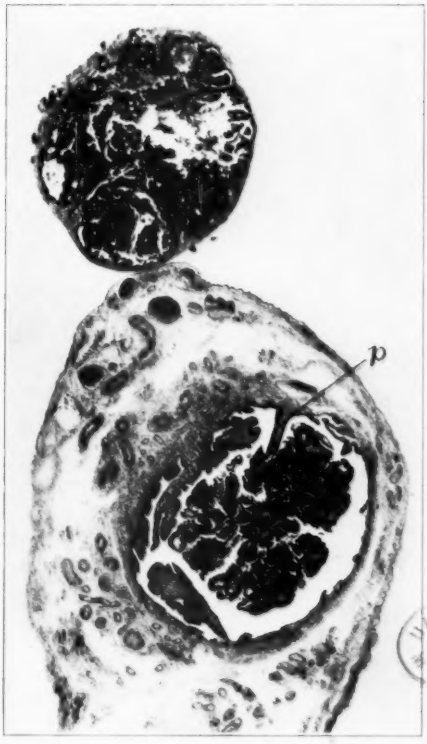


100

Sampson

Carcinoma of Tubes and Ovaries

Plate 36

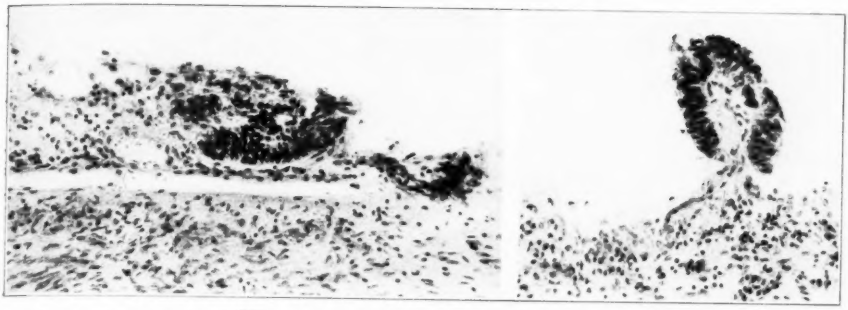
Fig. 101. Photomicrograph of an early implantation of cancer cells on the surface of the ovary between "b" and "c" of Fig. 98. Serial sections made of this portion of the block demonstrated that the carcinoma shown here was not continuous with that elsewhere. It represents a section of the largest part of the growth. A typical reaction to a foreign body is shown; the cancer cells in the center are enmeshed in newly formed tissue, which has arched over the surface of the ovary, while those to the right are becoming encapsulated on the surface of the ovary. Cancer cells might have escaped from any of the nearby exposed peritoneal implantations and lodged on the surface of the ovary in this situation. A later stage of the condition shown here might resemble the growth shown in Fig. 103, and also in Figs. 91 and 92. × 130.

Fig. 102. Photomicrograph of an early implantation on the upper surface of the mesosalpinx shown in Fig. 98 from another section of the same block. This too is not continuous with carcinoma elsewhere. It is non-encapsulated and is either an implantation of grafted type or the result of a differentiation of the mesothelium in this situation. The former theory appeals to me as being the more likely. A later stage of this implant might resemble those shown in Figs. 93, 100 and 104. Compare also with Figs. 21, 36, 39, 51 and 65. × 130.

Fig. 103. Photomicrograph (higher magnification) of carcinoma on the surface of the left ovary, indicated by "a" of Fig. 98. It might have begun as shown in Fig. 101 and subsequently spread over the surface of the ovary. If not sufficiently checked by encapsulation implantations similar to those shown in Figs. 96, 101 and 102 might arise from it. One can see that clumps of cancer cells would easily escape into the peritoneal cavity from the surface of such an implant and might give rise to secondary implantations of the growth. × 54.

Fig. 104. Photomicrograph (higher magnification) of implant "c" of Fig. 98, but from another section of the block. One can realize that this might have begun as the implant shown in Fig. 102. Compare also with Fig. 95. × 25.

Fig. 105. Photomicrograph of a portion of the surface of one of the tubal carcinomas shown in Fig. 96, and also of the tubal mucosa opposite it. Is the carcinoma of the mucosa a contact implant or one of multicentric origin? I was unable to demonstrate that it was continuous with the growth above it. × 54.



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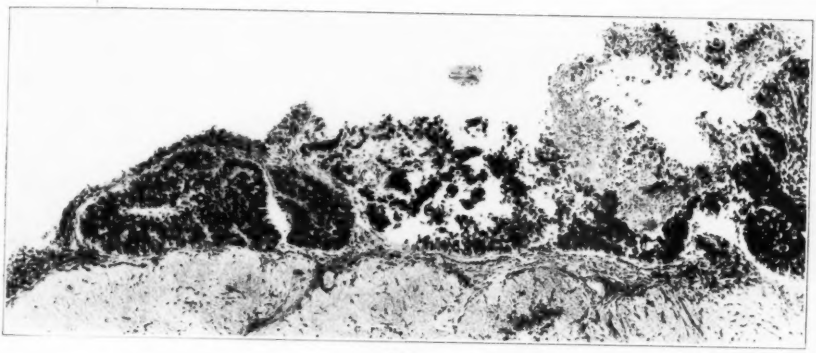

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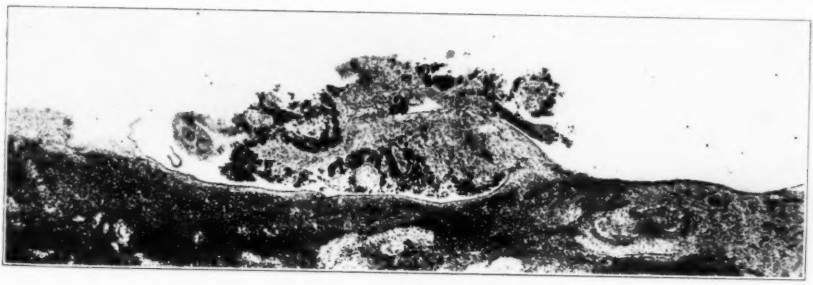

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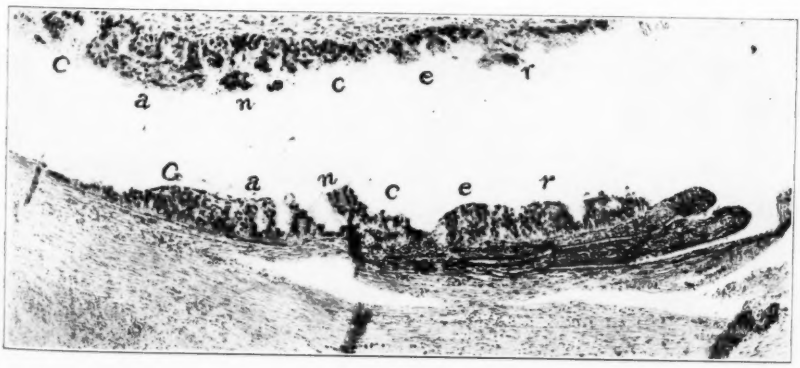

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PLATE 37

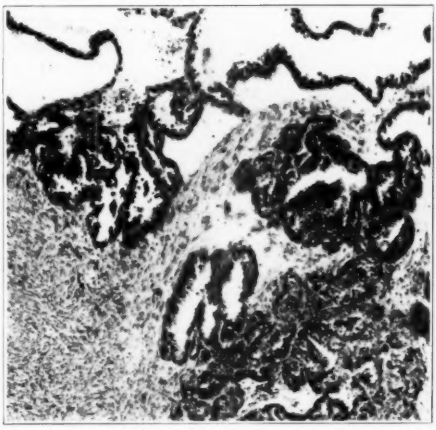
FIG. 106. Photomicrograph of a section of a portion of the uterine mucosa from a patient (Case 15) with adenocarcinoma of the body of the uterus treated with radium 2 months before. Carcinoma is still present in the deeper portion of the endometrium. This was found only in a few places. Carcinoma was not found in the uterine wall (many sections from several blocks were examined). × 54.

FIG. 107. Photomicrograph of a section of a portion of the right ovary showing a judged metastatic adenocarcinoma of that organ secondary to that of the uterus. What is the pathogenesis of this carcinoma of the ovary? This photomicrograph is of a portion of the wall of the small ovarian cyst shown in the next illustration, but from another section of the series. × 54.

FIG. 108. Photomicrograph of a cross-section of the right tube and ovary. The tube appears normal. It was patent, but presented no particles of carcinoma in its lumen. Carcinoma, apparently in patches "c," "c," "c," "c," is scattered on the surface of the ovary. A small malignant ovarian cyst is present, the epithelial lining of which is continuous with that of the growth on the surface of the ovary. Carcinoma is enmeshed in newly formed tissue which has arisen on the surface of the ovary between it and the tube. Serial sections demonstrated that the majority, if not all, of the patches of carcinoma on the surface of the ovary composed one tumor which had spread over the surface of that organ in an irregular manner. Carcinoma was not found, either in the lymph vessels of the mesosalpinx, or hilum of the ovary. The distribution and general histological picture of the ovarian carcinoma in this case are exact duplications of those frequently encountered in ovarian endometriosis. × 5.

FIG. 109. Photomicrograph of carcinoma enmeshed in newly formed tissue on the surface of the ovary. What is its pathogenesis? Serial sections demonstrated that it was but a part of the advancing edge of the growth in the newly formed tissue on the surface of the ovary between it and the adherent tube of the preceding illustration. × 54.

106



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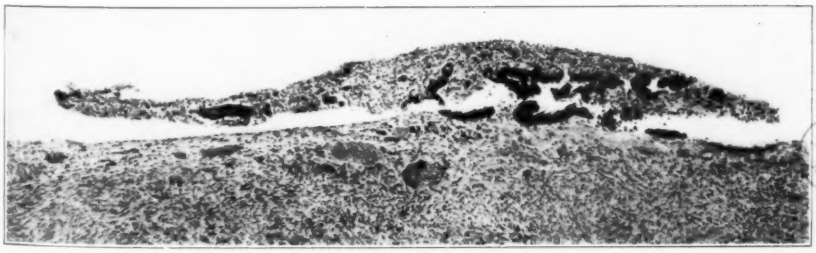

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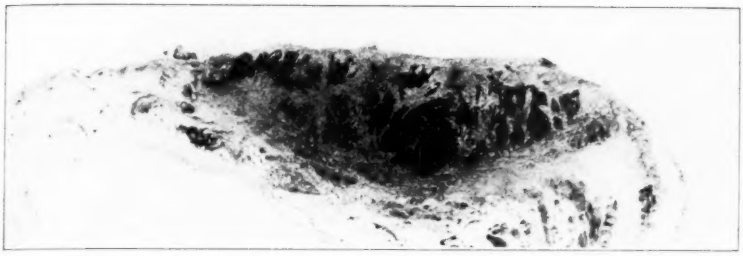
PLATE 38

FIG. 110. Photomicrograph of a section of a peritoneal metastasis in the posterior cul-de-sac. Its general histological structure is similar to that of encapsulated foreign bodies in the peritoneum and likewise that of many of the peritoneal metastases in ovarian carcinoma and of peritoneal endometriosis. The growth in this situation had invaded some of the subperitoneal lymphatics as a primary carcinoma invades the lymphatics of the organ in which it arises. × 10.

FIG. 111. Photomicrograph of a section of a portion of the anterior wall of the uterus and (to the right) the uterovesical reflection of peritoneum. The surfaces of both are covered with carcinoma. At operation the uterovesical reflection of peritoneum was found to be fused with the lower portion of the anterior wall of the body of the uterus. During the operation the adherent peritoneum was pulled away from the anterior wall of the uterus, thus exposing the carcinoma which had developed in the bottom of the anterior cul-de-sac and fused its peritoneum to the anterior wall of the uterus. Similar conditions are frequently found in this situation in both peritoneal carcinomatosis of ovarian origin and in peritoneal endometriosis. × 5.

FIG. 112. Photomicrograph of a cross-section of the tip of an epiploical appendage of the sigmoid, which was fused with the peritoneum of the posterior cul-de-sac by carcinoma uniting the surfaces of the two structures. They were separated at the operation. The appendage and the corresponding area of the peritoneum were excised. What is the pathogenesis of these multiple patches of carcinoma on the surface of the ovary and peritoneum? The capsules containing radium, which were introduced through the cervix into the uterine cavity like the plunger of a piston syringe, easily could have forced blood-containing particles of cancer out through the patent tubes into the peritoneal cavity. The subsequent implantation of these particles of cancer would account for the metastases found at operation 2 months after the application of radium. × 10.

FIG. 113. Photomicrograph of a section of the metastasis found in the scar of the abdominal incision 4 months after the uterus, tubes and ovaries were removed, and the peritoneal implants shown in the preceding illustration were excised. The metastasis is situated just beneath the skin, where the edges of the abdominal wall were approximated in the closure of the abdominal incision. Particles of cancer might easily have been implanted in the abdominal wound during the excision and removal of the peritoneal metastases. There was no induration of the abdominal wall beneath the scar. The strongest possible circumstantial evidence indicates that this metastasis arose from the successful implantation of cancer in the wound of the abdominal incision during the operation, just as the other judged implantation metastases shown in this paper arose from the lodging and retention of cancer cells in a wound, with the attempted healing of the wound and the continued growth of the carcinoma in its new situation. × 6.



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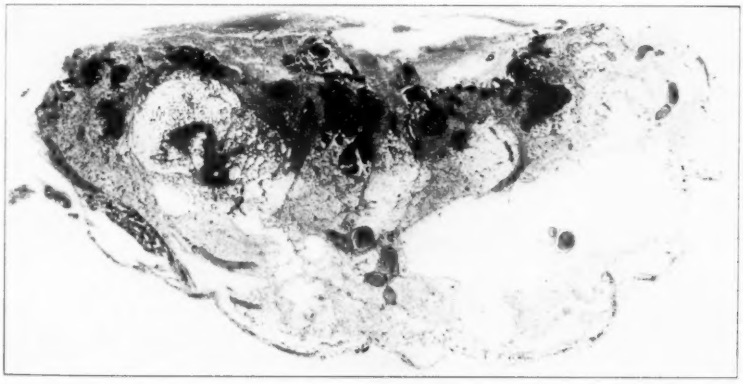

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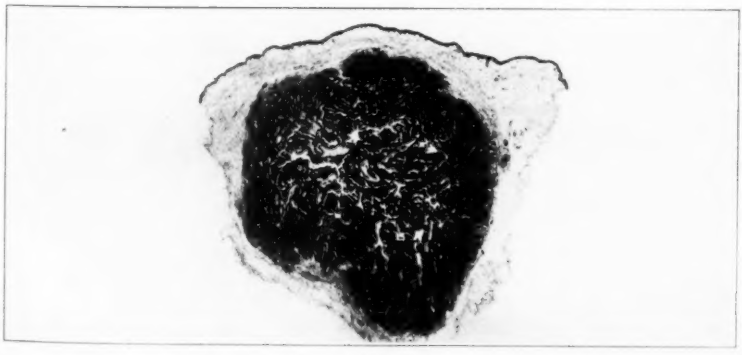

113

STUDIES ON THE MATURE AND IMMATURE LYMPHOID CELLS OF SPLEEN, LYMPH NODES AND THYMUS OF NORMAL RATS AND RATS INFECTED WITH TRYPANOSOMA BRUCEI *

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In a previous histological study on the lymphatic reaction in experimental trypanosomiasis † it was found that in the spleen and lymph nodes of white rats infected with *Trypanosoma brucei* there was, besides increased activity in the hematopoietic and reticulo-endothelial systems, a very striking lymphoblastic hyperplasia. Many of the large lymphoblasts that appeared in the early stage of infection were found to be formed by hypertrophy of the small round cells (ordinarily known as small lymphocytes), which constitute the main bulk of these organs. Not all of the small round cells, however, underwent hypertrophy, many of them remaining small even at the end of infection. Furthermore the small round cells of the thymic

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† Since the original report may not be easy of access to all readers of this article, an abstract is given here.

In the sections of normal rat spleen, lymph nodes and thymus, stained with Mallory's eosin-methylene blue stain, five varieties of cells can be differentiated as follows: (1) Large and medium sized lymphoblasts with intensely basophilic cytoplasm and clear nuclei with large irregular chromatin masses. These cells are found not only in the germinal centers, but also scattered among the small lymphoid cells. (2) Large and medium sized lymphocytes with only faintly basophilic cytoplasm. These are also widely scattered, but are especially numerous in the outer zones of the lymph follicles of the spleen. (3) Small lymphoid cells with very scanty cytoplasm and small round nuclei. These form the main bulk of the lymphoid organs. (4) Plasma cells with violet cytoplasm and eccentric nuclei. These are absent in the splenic lymph follicles of normal rats but are very numerous in the medulla of the lymph nodes — especially those of the chest and abdominal cavities. (5) Cells of the reticulo-endothelial system, which have acidophilic cytoplasm, fine nuclear chromatin masses and thin nuclear membrane, and can therefore be distinguished easily from all the above varieties.

In the experiment the rats, after they were each inoculated intraperitoneally with about 10,000 trypanosomes, died in from 7 to 10 days. By killing them on successive days after inoculation it was possible to follow the series of changes taking place in the lymphoid organs. Forty-eight hours after inoculation the spleen was found to be already greatly enlarged, and in sections many large lymphoblasts had appeared in the interfollicular lymphoid tissue where normally only a few such cells were present. At this stage it was also found that there were many intermediate forms between the large lymphoblasts and the small lymphoid cells. Especially significant was the scarcity of mitotic figures and the slightly increased amount of dark blue cytoplasm around the

cortex, which are also considered by many to be small lymphocytes, did not hypertrophy. We therefore assumed that these small round cells, which we designated as small lymphoid cells, could be divided into two types, according to their ability to hypertrophy: one, the small primitive cells which can form large lymphoblasts; the other, the true small lymphocytes which cannot form large lymphoblasts. Since these two types cannot be differentiated from each other in sections stained by the usual methods, the present study was made in an attempt to differentiate them by using the supravital technique.

Material and Methods

White male rats from 5 to 6 months old, raised in China, were used. Occasionally, younger animals were employed. The strain of *Trypanosoma brucei* was kindly supplied by Professor F. G. Novy. A preliminary study showed that the histological pictures of the normal and the infected rats were identical with those previously described.[1]

The infective material consisted of citrated blood of an infected rat, diluted 10 to 20 times with normal saline. Each animal to be infected was injected subcutaneously or into the thymus with an amount of the material containing approximately 10,000 or 3000 parasites respectively. In intrathymic injections a small amount of India ink was added to the inoculum as an indicator, and in order to facilitate these injections smaller animals 2 to 3 months old were used.

The cells studied were those of the inguinal lymph nodes, the mesenteric lymph nodes, the spleen and the thymus. Since the inguinal lymph nodes are easy of access and the thymus cannot be exposed without endangering the life of the animal, the inguinal lymph nodes were examined first, then the abdominal lymphoid organs and finally the thymus. All operations were carried out under ether anesthesia. A small bit of the desired tissue was removed with a pair of fine scissors and immediately placed in a drop of rat serum on a clean coverslip, where the cut surface of the tissue was gently scraped with a cataract knife until the serum became slightly cloudy. A small drop of the serum was then trans-

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nuclei of some of the small lymphoid cells. The increase of the lymphoblasts at this stage then, was apparently due to the hypertrophy of some of the small lymphoid cells. There were, however, many small lymphoid cells in which no change could be detected.

During the next 2 or 3 days there was a progressive increase in number of both the large, and especially the medium sized lymphoblasts, while the unchanged small lymphoid cells gradually decreased in number. Mitotic figures among the lymphoblasts became very numerous. The plasma cells and the transitional forms between the lymphoblasts and plasma cells also began to appear in the lymph follicles in large numbers. After the 6th day the spleen became less cellular, owing to the reduction in number of the lymphoblasts and plasma cells.

The above described changes were most striking in the spleen and in the abdominal and thoracic lymph nodes. The inguinal lymph nodes showed only moderate lymphoblastic hyperplasia. The thymic cortex showed surprisingly little change, in spite of the fact that marked lymphoblastic hyperplasia was present in the lymph nodes in its immediate neighborhood.

ferred to another coverslip, which was instantly placed on a slide previously coated with neutral red and Janus green. From this film 500 cells were immediately counted. Just before opening the chest cavity to expose the thymus the animal was exsanguinated from the carotid artery. For the cell suspension of infected rats the serums of both normal and infected animals were used, with no apparent difference in results.

Description of the Lymphoid Cells

It may be said at the outset that the lymphatic change observed in experimental trypanosomiasis of the rat is largely of a quantitative nature, involving the different types of lymphoid cells found under normal circumstances. The following tabular description of the cells (see Table I) is given as a basis for the presentation of the observations under discussion.

Observations on Normal Rats

As a control to the findings obtained on the cells of the lymphoid organs of rats infected with *Trypanosoma brucei* parallel observations were made on normal rats of the same age and sex, fed on the same diet. Before referring to the differential counts in this control series the following points should be mentioned as possible sources of error in work of this type.

1. The mitochondria, which are usually well stained within a few minutes after the film is made, sometimes lose their stain very rapidly without the occurrence of any other noticeable morphological change. The loss of stain is found to be due to overcrowding, for in the films with fewer cells this phenomenon is not observed.

2. Most of the degenerated cells (Fig. 19) are originally small immature lymphocytes. The degenerative change takes place so rapidly that unless the films are examined quickly it will not be observed. In the process of degeneration a clear zone first appears around the nucleus; then the rest of the cytoplasm swells so that it becomes practically invisible. While this swelling of the cytoplasm is taking place the mitochondria, which are sometimes already stained, also swell and begin to dance about, and finally become stainless granules, or what look like vacuoles either diffusely scattered or grouped together on one side of the nucleus, which, in the meantime, has become round and homogeneous but unaltered in its size. The exact cause of the degeneration is not known and the number of degenerated cells does not seem to be appreciably in-

TABLE I

Description of Different Types of Lymphoid Cells Found in the Rat

Cell name	Size [3]	Cytoplasm	Mitochondria	Neutral red bodies [6]	Nucleus
Lymphoblasts	L,[4] M–S, S	Opaque	Coarse and abundant	None or very few	Rounded or irregular, large chromatin masses
Immature lymphocytes	L, M–S, S	Slightly opaque	Medium sized, less abundant	Few	Rounded or irregular
Lymphocytes	L, M–S, S	Clear	Fine, scanty	Few or numerous	
Degenerated cells	L, M–S, S [5]	Swollen, practically invisible	Usually not seen, but there are unstained vacuoles		Round, homogeneous, nuclear membrane distinct
Plasma cells [1]	L, M–S, S	Opaque	Coarse, medium sized or fine	Few and fine	Usually rounded, eccentric position, large chromatin masses
Monocyte-like cells [2]	L, M–S, S	From opaque to clear	From coarse and abundant to fine and scanty	Few or abundant, rosettes	Kidney-shaped, eccentric position

1. There are in the infected animals cells representing the transitional forms between lymphoblasts and plasma cells. Such forms are classified either as lymphoblasts or plasma cells, depending upon the type to which they show the greatest resemblance.

2. It is notoriously difficult in the rat to differentiate monocytes from lymphoid cells. The term "monocyte-like" employed here is used merely to indicate a group of cells having certain morphological characteristics without definitely implying that they belong to another cell strain.

3. The size of the cell is classified as large (L) if the cell diameter is over 12 microns, medium sized (M–S) if it is between 9 and 12 microns, and small (S) if it is between 7 and 9 microns.

4. Some large lymphoblasts may measure 20 or more microns in diameter.

5. Owing to the marked swelling of its cytoplasm the size of the degenerated cell is determined not by its diameter but by the size of its nucleus, using the nuclei of the undegenerated lymphoid cells for comparison. According to this method of determination it was found that most of the degenerated cells were of the small variety.

6. There is a great variation in the amount and arrangement of the neutral red bodies in different cells, which is especially noticeable in the lymphocytes. A classification of the cells according to the arrangement of their neutral red bodies will be given in the following paper [2]; but as they are found only in small numbers in this study no further differentiation will be made here.

fluenced, either by mechanical pressure, by the action of the stains, or by the age of the preparation.

3. Although many of the small round cells of the thymus are indistinguishable from those of the lymph nodes and spleen there is,

TABLE II

Average Percentage Values of the Differential Counts on the Organs of 7 Normal Rats: 500 Cells Counted in Each Preparation

Type of Cell	Organ			
	Inguinal lymph node	Mesenteric lymph node	Spleen	Thymus
Small lymphoblasts	0.6	1.3	2.0	1.1
Small immature lymphocytes	75.7	64.7	60.0	83.9[2]
Small lymphocytes	0.9	1.9	1.9	+
Small degenerated cells	21.2	29.3	28.9	9.7
Medium sized lymphoblasts	0.3	0.4	1.3	2.0
Medium sized immature lymphocytes, lymphocytes and degenerated cells[1]	0.3	1.0	2.7	0.9
Large lymphoblasts	0.2	0.4	0.8	1.1
Large immature lymphocytes, lymphocytes and degenerated cells[1]	0.2	0.3	0.9	0.5
Plasma cells	0	0.4	0.3	+
Monocyte-like cells	+	0	0	0
Clasmatocytes	0.1	0.1	0.6	0.4
Neutrophilic leukocytes	0.1	0.1	0.4	0.1
Eosinophilic leukocytes	+	+	0.3	+

1. Owing to their scarcity these cells are grouped together under their respective sizes.
2. Includes some cells morphologically slightly different (see text).
+ = present, but less than 0.1 per cent.

among the small immature lymphocytes, one type that is much more frequently seen in this organ than in the other organs. Cells of this type (Figs. 10, 11, 12) are characterized by rather deep and coarse neutral red bodies, usually two or three in number, but sometimes more, generally clustered on one side of the cell. The mitochondrial

content is not noticeably different from that of the ordinary small immature lymphocytes.

Differential counts of the cell suspensions from the surface scraping of the inguinal lymph nodes, mesenteric lymph nodes, spleen and thymus of 7 normal animals were made, the results of which are shown in Table II. It will be seen that lymphoblasts of all sizes (Figs. 1, 20, 27) were found in all these organs, although only in small numbers. The majority were the small immature lymphocytes (Figs. 3, 4, 5 and 6) which, together with their degenerated forms, constituted about 90 per cent of the total count. Attention is to be given to the fact that whereas the percentage of small degenerated cells was fairly high in the lymph node and the spleen films, it was much lower in those of the thymus. Mention should also be made of the fact that although myeloid cells were occasionally seen in the films made from splenic tissue, they were not encountered when these particular counts were made.

Observations on Infected Rats

It has been found [1] that intraperitoneal injection of *Trypanosoma brucei* is followed by a rapid lymphoblastic hyperplasia of the spleen and of the lymph nodes of the thoracic and abdominal cavities. The reaction in the inguinal and other superficial lymph nodes, however, is only slight or moderate. The absence of a marked reaction in these lymph nodes is apparently due to the scarcity of parasites in them. To prove this point 7 rats were infected subcutaneously in the right inguinal region. On the 7th day after inoculation it was found that the spleen was as much enlarged as in those animals infected by the intraperitoneal route, but also that there was an extreme enlargement of the inguinal lymph nodes on the side of injection (Fig. 32). They formed a mass, fifteen or twenty times larger than normal, and areas of necrosis and hemorrhage were frequent. In marked contrast to this finding the inguinal lymph nodes on the left side, which was not inoculated, and the thoracic and mesenteric lymph nodes showed only slight enlargement. The results of the cell counts on these animals are shown in Table III.

In Table III it will be noted that the increase in the number of lymphoblasts and plasma cells is most marked in the right inguinal lymph nodes, slightly less in the spleen, much less in the mesenteric

and left inguinal lymph nodes, and almost unnoticeable in the thymus.

The differences between the percentage values of the lymphoblasts and plasma cells of the lymphoid organs of the normal rats

TABLE III

Average Percentage Values of the Differential Counts on the Organs of 7 Infected Rats Examined on the 7th Day of Infection: 500 Cells Counted in Each Preparation

Type of Cell	Organ				
	Right inguinal lymph node	Left inguinal lymph node	Mesenteric lymph node	Spleen	Thymus
Small lymphoblasts	13.3	0.8	1.7	6.0	1.3
Small immature lymphocytes	39.0	66.1	70.0	49.4	82.0[2]
Small degenerated cells	19.1	29.0	22.9	13.8	11.2
Small lymphocytes	0.3	0.1	0.5	0.4	0
Medium sized lymphoblasts	9.8	0.9	1.0	8.3	2.4
Medium sized immature lymphocytes, lymphocytes and degenerated cells[1]	0.9	0.6	0.3	0.9	0.4
Large lymphoblasts	7.0	0.3	0.5	7.9	1.1
Large immature lymphocytes, lymphocytes and degenerated cells[1]	1.0	0.3	0.3	1.9	0.2
Plasma cells	6.5	0.2	1.6	4.2	+
Monocyte-like cells	+	0.2	0	+	+
Clasmatocytes	2.9	1.4	0.8	6.1	1.0
Neutrophilic leukocytes	0.2	0.1	0.1	0.7	0.3
Eosinophilic leukocytes	+	0.3	0.2	0.5	+

1. Owing to their scarcity these cells are grouped together under their respective sizes.
2. Includes some cells morphologically slightly different (see text).
+ = present, but less than 0.1 per cent.

and those of the infected rats, as shown in Tables II and III, are graphically represented in Chart I.

The striking difference in the reaction of the inguinal lymph nodes of two sides indicates that the degree of hyperplasia in the lymphoid tissue is dependent upon the local concentration of parasites. In

order to ascertain if the failure of the thymus to participate in the general reaction is also due to the absence in it of parasites, a mixture of India ink and about 3000 trypanosomes was injected directly into the thymus of 5 rats. By the 5th or 6th day, when the blood was swarming with trypanosomes and the animals were in a moribund state, counts of thymic cells were made. The results of these counts, as shown in Table IV, indicate that lymphoblastic proliferation was

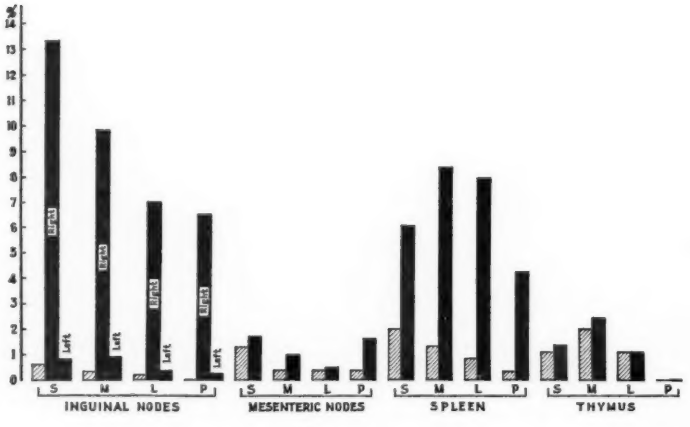

CHART 1

A graphic representation of the percentage values of lymphoblasts and plasma cells in lymphoid organs of the normal and infected rats.

Shaded columns = normal animals. Black columns = infected animals. S = small lymphoblasts. M = medium sized lymphoblasts. L = large lymphoblasts. P = plasma cells.

negligible. In addition, microscopic examination of sections of thymus showed only a very slight lymphoblastic hyperplasia; practically all the cortical cells were small cells (Fig. 37). The presence of a large amount of India ink demonstrated that trypanosomes had been actually introduced into the gland (Fig. 36). In contrast, it was of especial interest that in each animal the mediastinal lymph nodes in close proximity to the thymus presented a picture of marked lymphoblastic or plasma cell hyperplasia (Figs. 38 and 39).

TABLE IV

Average Percentage Values of Differential Counts of the Thymus of 7 Normal and 5 Infected Rats: 500 Cells Counted in Each Preparation.

Type of Cell	Normal	Infected
Small lymphoblasts	1.1	2.0
Small immature lymphocytes	83.9[2]	82.7[2]
Small degenerated cells	9.7	9.7
Small lymphocytes	+	0
Medium sized lymphoblasts	2.0	3.0
Medium sized immature lymphocytes, lymphocytes and degenerated cells[1]	0.9	0.6
Large lymphoblasts	1.1	1.2
Large immature lymphocytes, lymphocytes and degenerated cells[1]	0.5	0.1
Plasma cells	+	0.3
Monocyte-like cells	0	0.1
Clasmatocytes	0.4	0.4
Neutrophilic leukocytes	0.1	0.1
Eosinophilic leukocytes	+	0

1. Owing to their scarcity these cells are grouped together under their respective sizes.
2. Includes some cells morphologically slightly different (see text).
+ = present, but less than 0.1 per cent.

DISCUSSION

The characteristic and striking increase of large and medium sized lymphoblasts of the spleen and lymph nodes of rats infected with *Trypanosoma brucei* has again been demonstrated in this experiment. In previous experiments this information was obtained by means of tissue fixed in the usual way, but in the observations here reported the supravital technique was employed.

In this study we find three criteria of considerable value in judging the age of these cells. They are the degree of opacity of the cytoplasm, the number of neutral red bodies, and the number and size of

mitochondria.* Taking these three criteria together we have been able to differentiate the lymphoid cells into three main groups — lymphoblasts, immature lymphocytes and lymphocytes. The possibility of differentiating the small lymphoid cells into these three groups further confirms our previous assumption that failure of some of the small lymphoid cells to hypertrophy is due to the maturity of the cells.

In certain instances, however, a clear-cut classification may not be possible, as in a small cell where scarcity of cytoplasm makes it difficult to estimate its opacity. In such a case the character of the mitochondria and the presence or absence of neutral red bodies are the important points that determine the degree of maturity. On the other hand, increased opacity of the cytoplasm alone is not always dependable for the identification of young cells; for plasma cells (Figs. 18, 25), which are end products as far as further proliferation is concerned, possess cytoplasm as opaque as that of the lymphoblasts. Nor does the presence of neutral red bodies always signify maturity; for lymphoblasts, while in the circulation, or in the presence of some abnormal irritant, can exhibit conspicuous neutral red bodies without loss of the function of proliferation.

Since they represent a series of transitional stages between the typical small lymphoblasts and the typical lymphocytes, the small immature lymphocytes necessarily vary in the degree of maturity. There is no satisfactory evidence to prove that some of the younger forms of this group can hypertrophy like the typical small lymphoblasts, but such a possibility is suggested by the fact that the number of the newly formed large lymphoblasts in the sections of the spleen and lymph nodes examined 48 hours after inoculation is larger than that of the small lymphoblasts normally existing in these organs, as shown in Table II.

The present experiments support the following opinion on the origin of the plasma cells, formed on the basis of the previous histological study in experimental trypanosomiasis. During the terminal phase of the infection, when medium sized and small lymphoblasts are formed in great numbers as a result of repeated and rapid mitotic

* After the completion of the present investigation in 1931 our attention was called to the work of Wiseman [3] who used the basophilia of the cytoplasm and the mitochondria of the cell as criteria for the determination of the age of the cells in the peripheral blood. It is interesting to note that his and our methods of classification of the lymphoid cells are essentially in agreement.

division of larger lymphoblasts, there is also a rapid and concomitant appearance of the plasma cells. The presence of many transitional forms between typical lymphoblasts and typical plasma cells (Figs. 14, 15, 16, 17, 23, 24, 29, 30 and 31) is very suggestive of the lymphoblastic origin of plasma cells.*

With respect to the thymus, it will be remembered that the origin of the cortical cells is still under discussion. Some investigators believe in their epithelial origin,[5,6,7] and others derive them from lymphocytes.[8,9] From the morphological point of view most of the thymic cells in the supravitally stained films are indistinguishable from lymphoid cells, but some of them contain several conspicuous deep red bodies which are only occasionally seen in the lymphoid cells from other lymphoid organs. This feature, however, does not of itself seem sufficient evidence for the assumption that they are different from lymphoid cells. The morphological differences among the ordinary lymphoid cells themselves may sometimes be even greater. On the other hand, Table II shows 9.7 per cent of degenerated cells in the films from thymus and 21.2 to 29.3 per cent of similar cells in those from other organs. This suggests that there is an actual physiological difference. Contributary evidence on this point is furnished by the failure on the part of the thymic cortical cells to show a hyperplastic reaction after the direct injection of trypanosomes into the organ. The failure to react is not due to a lack of young forms, as differential counts have shown. A similar condition is found in cases of lymphatic leukemia in which all the lymphoid organs, except the thymus, are enlarged.[6,10] This difference in behavior suggests that the thymic cortical cells are not of lymphoid origin, or, if they are, that conditions prevailing in the thymus successfully prevent them from reacting in the manner of lymphoid cells, as in other lymphoid organs.

* Recently Miller[4] has demonstrated the primitive connective tissue cells as the origin of plasma cells in rabbits following repeated injections of tuberculo-proteins. He did not specify the time interval between the first injection and autopsy. But if we assume that the injections were made once daily, the average of this interval would be at least 17 days. Since it has been demonstrated in our experiments that lymphoblasts can proliferate and transform into plasma cells in 6 or 7 days, it seems that his observations were made too late to exclude definitely the possibility of lymphoblastic origin of the plasma cells.

Summary

The large, medium sized and small lymphoid cells of the spleen and lymph nodes of normal rats can be classified by means of the supravital technique as lymphoblasts, immature lymphocytes and lymphocytes. The proportions of these different types of cells are found to be altered in the rats infected with *Trypanosoma brucei* by the increase in the percentage of lymphoblasts and plasma cells. The assumption made in the previous report [1] that the initial appearance of many large lymphoblasts in the lymphoid organs of the infected animals is due to hypertrophy of the small "primitive cells" (lymphoblasts) receives confirmation from the results of the present study. These hypertrophic lymphoblasts produce by mitosis other lymphoblasts, which later transform into plasma cells. The lymphocytes and most of the immature lymphocytes have not been found capable of hypertrophy or mitotic division.

Functionally the cortical cells of thymus are different from the small lymphoid cells of the spleen and lymph nodes.

The classification of the different types of lymphoid cells permits of both qualitative and quantitative studies of cells of lymphoid tissues under normal and pathological conditions.

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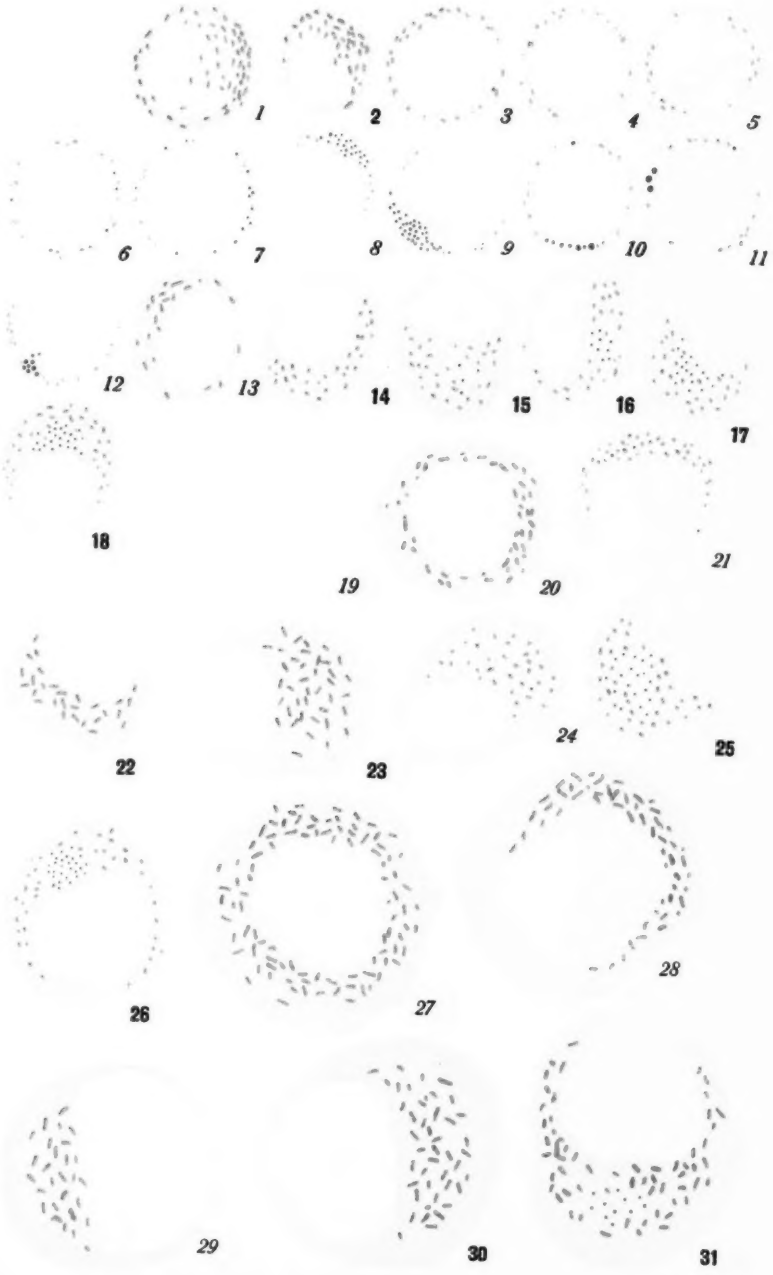
DESCRIPTION OF PLATES

PLATE 39

The cells illustrated in this plate were drawn within 10 minutes after the films of the organs were made according to the technique described in the text. They were from the spleen, lymph nodes (inguinal and mesenteric), and thymus of 2 normal and 2 infected rats. Cells from infected animals have their numbers printed in arabic type; those of the normal animals in italics. The size of a red blood cell is included for comparison.

The following types of cells are illustrated:

Small lymphoblasts — 1, 2, 13.
Small immature lymphocyte, younger form — 3.
Small immature lymphocytes, older form — 4, 5, 6.
A type of small immature lymphocytes of thymus — 10 to 12.
Small lymphocytes — 7 to 9.
Transitional forms between small lymphoblasts and typical plasma cells — 14 to 17.
Typical small plasma cell — 18.
Small degenerated cell — 19.
Medium sized lymphoblasts — 20, 22.
Medium sized immature lymphocyte — 21.
Transitional forms between medium sized lymphoblasts and plasma cells — 23, 24.
Medium sized plasma cell — 25, 26.
Large lymphoblasts — 27, 28.
Transitional forms between large lymphoblasts and plasma cells — 29 to 31.
Cells from inguinal lymph nodes of normal rat — 4, 6, 7, 19.
Cells from mesenteric lymph nodes of normal rat — 8, 20.
Cells from spleen of normal rat — 1, 3, 27.
Cells from thymus of normal rat — 5, 28.
Cells from inguinal lymph node of infected rat — 2, 14, 15, 16, 17, 18, 22 23, 25, 26, 30, 31.
Cell from mesenteric lymph nodes of infected rat — 21.
Cells from spleen of infected rat — 13, 24.
Cell from thymus of infected rat — 11.



Hu

Lymphoid Cells of Spleen, Lymph Nodes and Thymus

PLATE 40

FIG. 32. Low power view of the right inguinal lymph node of Rat 165, which was injected subcutaneously in the right inguinal region with about 10,000 trypanosomes and killed on the 7th day. × 11.

FIG. 33. Low power view of the left inguinal lymph node of the same rat (No. 165). It is much smaller than the node shown in Fig. 1. × 11.

FIG. 34. High power view of the cortical tissue of the right inguinal lymph node in the area marked "A" in Fig. 1. Numerous large lymphoblasts are seen. One cell is undergoing mitosis. Hematoyxlin and eosin stain. × 800.

FIG. 35. High power view of the cortical tissue of the same lymph node in the area marked "B" in Fig. 1, showing lymphoblasts, plasma cells and intermediate forms between the lymphoblasts and plasma cells. Hematoxylin and eosin stain. × 800.

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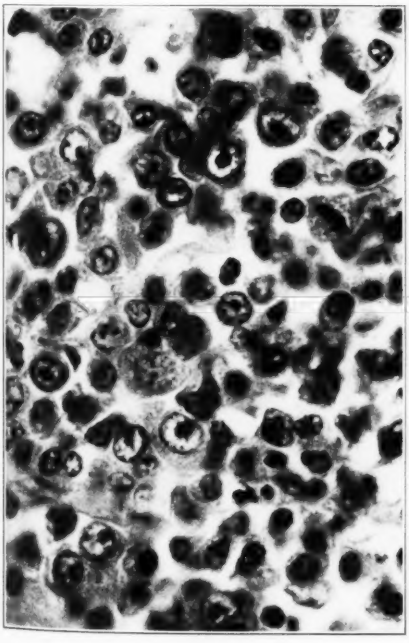

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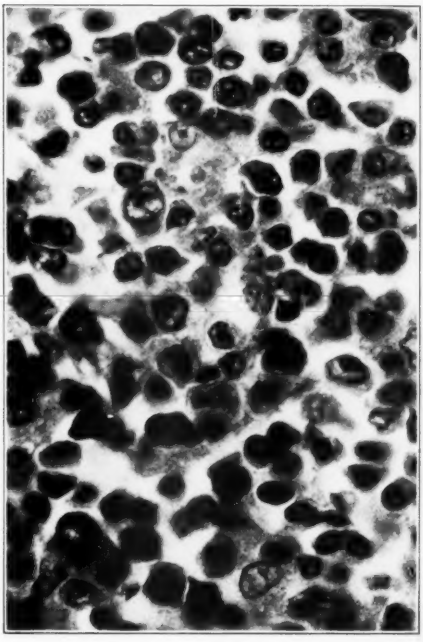

35

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Lymphoid Cells of Spleen, Lymph Nodes and Thymus

PLATE 41

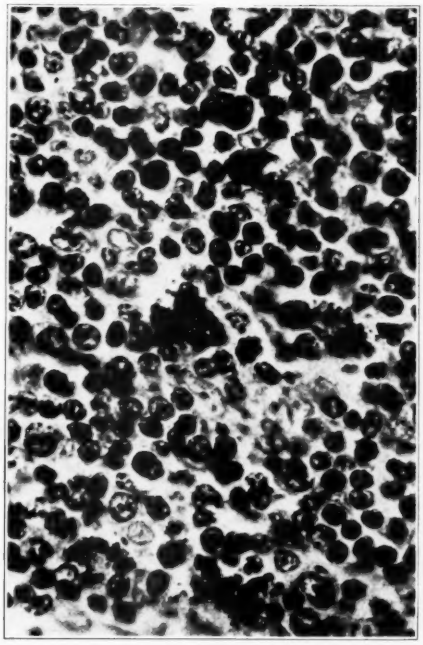
FIG. 36. Low power view of thymus (T) and mediastinal lymph node (L) of Rat 186. This animal was injected intrathymically with about 3000 trypanosomes mixed with India ink (seen in this illustration as intensely black masses or particles), and was then killed on the 5th day of infection. Section lightly stained with hematoxylin. × 9.5.

FIG. 37. High power view of an area in the thymic cortex, indicated by "A" in Fig. 5, but from the next serial paraffin section. Masses of ink present in the tissue; no increase of large lymphoblasts seen. Mallory's eosin-methylene blue stain. × 800.

FIG. 38. High power view of an area in the mediastinal lymph node indicated by "B" in Fig. 5, but also from the next serial paraffin section. Many large lymphoblasts are present among the small lymphoid cells. Mallory's eosin-methylene blue stain. × 800.

FIG. 39. High power view of an area in the cortex of the mediastinal lymph node of Rat 187, which was injected intrathymically in the same manner as Rat 186 and killed on the 7th day of infection. Numerous plasma cells, and a few lymphoblasts and transitional forms between the plasma cells and lymphoblasts are seen. Hematoxylin and eosin stain. × 800.

36



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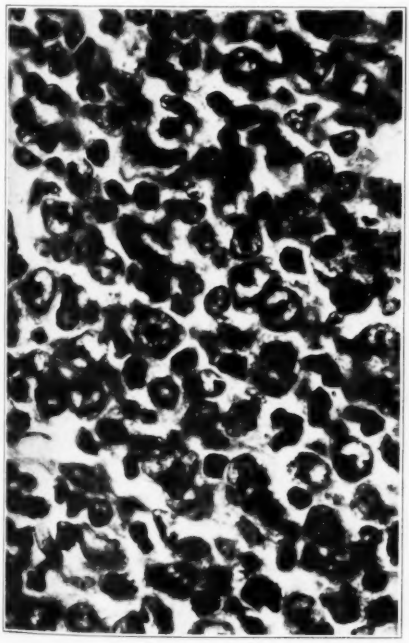

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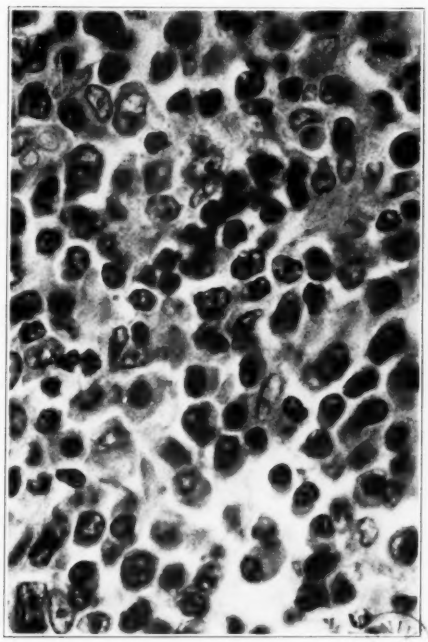

39

Hu

Lymphoid Cells of Spleen, Lymph Nodes and Thymus

STUDIES ON THE MATURE AND IMMATURE LYMPHOID CELLS OF THE PERIPHERAL BLOOD OF NORMAL RATS AND RATS INFECTED WITH TRYPANOSOMA BRUCEI *

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It has been shown in previous experiments[1,2] that lymphoblasts of all sizes are present in the diffuse lymphoid tissue of the spleen and lymph nodes of normal white rats, and that in animals infected with *Trypanosoma brucei* the small lymphoblasts in these organs hypertrophy to form large lymphoblasts, which in turn produce other lymphoblasts by mitosis. It has also been shown that in the latter part of the infection many plasma cells appear in the lymphoid organs as a result of direct transformation of the lymphoblasts.

In view of the remarkable lymphatic hyperplasia taking place in the spleen and lymph nodes of the infected rats, and because of the intimate relation between the lymphoid cells of the blood and those of the lymphoid organs, studies using the supravital technique were made on the blood cells of these normal and infected animals.

Material and Methods

Healthy male rats from 4 to 6 months of age, fed on a uniform diet, were used. The infected material consisted of citrated blood of an infected rat, diluted 10 to 20 times with normal saline. After a careful study of the blood of these animals they were each injected intraperitoneally with an amount of the material containing approximately 10,000 parasites.

Since it was necessary to secure perfectly fresh and clean drops of blood for supravital study and for cell counts, the usual methods of bleeding were found to be unsatisfactory. The cutting off the tip of the tail with scissors, or the severing of the tail veins with a razor, involves too much tissue injury and danger of secondary infection, especially when the animal is to be bled repeatedly in the course of the experiment; furthermore, the blood so obtained coagulates quickly and therefore is unsuitable for the purpose of counting. The puncturing of a deeply seated tail vein with a needle is not always successful. The veins of the feet, though easily accessible, are too small. The best are the saphenous veins, which are superficial and large. The skin on the inner surface of the thigh was shaved and thoroughly cleansed with alcohol and ether. The skin over the vein was drawn taut and held immobile so as to reduce the chance of hematoma formation. After the vein was punctured a drop of blood appeared on the skin

* Received for publication May 12, 1933.

surface. The size of the drop could be regulated, to a considerable extent, by the depth of the puncture and by the pressure applied on the vein proximal to the wound. When the desired amount of blood was obtained gentle pressure applied to the wound prevented further loss of blood or the development of a subcutaneous hematoma. By using the left and right veins alternately it was possible to bleed the animals for many days with very little loss of blood and a minimal amount of tissue injury. To prevent struggling, light ether anesthesia was used, which was found to produce no objectionable effect on the animals.

The trypanosomes, which may become very numerous in the blood, take up both Janus green and neutral red stains. Care must be exercised to use the correct quantity of stain so as to avoid overstaining and yet to ensure satisfactory demonstration of the structures of the cells.

Observations on the Blood of Normal Rats

The non-granular white blood cells of rats show, in supravitally stained films, great difference in size and opacity of their cytoplasm and in the number, size and arrangement of their mitochondria and neutral red bodies.

In order to evaluate the significance of these differences, a detailed classification such as that shown below is necessary. Code names are used for the sake of convenience.

SA_0	MA_0	LA_0	Deg.
SA_1	MA_1	LA_1	Mono.
SA_2	MA_2	LA_2	Plasma cell
SA_3	MA_3	LA_3	Clasmatocyte
SA_4	MA_4	LA_4	Myelocyte
SA_5	MA_5	LA_5	
SB_0	MB_0	LB_0	
SB_1	MB_1	LB_1	
SB_2	MB_2	LB_2	
SB_3	MB_3	LB_3	
SB_4	MB_4	LB_4	
SB_5	MB_5	LB_5	
SC_1	MC_1	LC_1	
SC_2	MC_2	LC_2	
SC_3	MC_3	LC_3	
SC_4	MC_4	LC_4	
SC_5	MC_5	LC_5	

Explanation

S = small (Figs. 1–37).
M = medium sized (Figs. 38–56).
L = large (Figs. 57–86).
A = lymphoblasts, characterized by opaque cytoplasm and coarse, abundant mitochondria (Figs. 1–9, 38–41, 57–65).
B = immature lymphocytes, representing the intermediate forms between lymphoblasts and lymphocytes, having less opaque cytoplasm; neutral red bodies generally present; mitochondria less coarse and less abundant than in the lymphoblasts (Figs. 10–20, 42–48, 67–75).

C = lymphocytes, characterized by clear cytoplasm and fine scanty mitochondria; neutral red bodies practically always present (Figs. 21–37, 49–56, 76–86).
0 = neutral red bodies absent (Figs. 1, 2, 38, 57, 58).
1 = few or numerous neutral red bodies diffusely arranged without any definite pattern; nucleus rounded or slightly irregular (Figs. 3–14, 19, 21–24, 29, 39, 42, 43, 49, 50, 59–62, 67–70, 76).
2 = neutral red bodies tend to concentrate in one part of the cell, usually in the slight concavity of the nucleus (Figs. 15–18, 26–28, 30, 40, 41, 44, 51–54, 63, 64, 77, 79).
3 = neutral red bodies concentrated in the indentation of the nucleus; nuclear indentation deeper (Figs. 31, 45).
4 = nucleus shows exaggerated indentation, so that it becomes V- or U-shaped, with the deep concavity filled by neutral red bodies (Figs. 32–35, 46, 47, 55, 56, 71–73).
5 = nucleus shows multiple indentations or folds, so that it may become actually lobulated. Neutral red bodies are distributed especially in the indentations or in the folds (Figs. 36, 37, 48, 74, 75, 78, 80–85).
Deg. = degenerated cells, each having a round, homogeneous, usually small nucleus, with unstained small vacuoles or granules usually situated on one side of it. Cytoplasm edematous or so swollen as to become invisible (Fig. 25).
Mono. = monocyte-like cells with kidney-shaped nuclei and rosettes of neutral red bodies. The term "monocyte-like" is used here to indicate cells having the above-mentioned characteristics, without implying that they belong to a cell strain different from that of the lymphoid cells (Figs. 87–90).
PMN = polymorphonuclear neutrophilic leukocytes.
PME = polymorphonuclear eosinophilic leukocytes.

In accordance with the above classification a total of 1000 white blood cells from twenty normal rats was counted each day on 6 different days. As the counts are fairly constant from day to day only the averages are shown in Table I.

The important points shown in this table are as follows. (1) In the peripheral blood of normal rats there are not only large lymphoblasts (total LA, 1.33 per cent) and medium sized lymphoblasts (total MA, 0.9 per cent), but also small lymphoblasts (total SA, 0.85 per cent). (2) Most of the blood lymphoblasts, in contrast to the lymphoblasts of the spleen and the lymph nodes, contain neutral red bodies. (3) There is a higher percentage of small lymphocytes in the peripheral blood (total SC, 23.11 per cent) than in the lymphoid organs (0.9 to 1.9 per cent), as shown in Table II of the preceding report.[2]

Observations on the Blood of Infected Rats

Red Blood Cells and Hemoglobin: In another set of 10 rats the number of erythrocytes and the percentage of hemoglobin were determined the day before, and then 7 days after they became

infected. A moderate anemia was found as a result of the infection (Table II).

Parasites and White Blood Cells: For the study of parasites and the white blood cells during infection we used the same group of 20 rats, whose normal white counts are shown in Table I. Two days after the last normal count was made each of these rats was inoculated intra-

TABLE I

Average Percentage Values of 6 Differential Counts (Done on April 2, 3, 4, 6, 7 and 9, 1931 Respectively) of the White Blood Cells of 20 Normal White Rats (Nos. 131 to 150)

SA0	0.03	MA0	0.10	LA0	0.17	Deg.	0.15
SA1	0.82	MA1	0.73	LA1	1.08	Mono.	2.33
SA2	0.00	MA2	0.02	LA2	0.03	Plasma cell	0.02
SA3	0.00	MA3	0.03	LA3	0.05	Clasmatocyte	0.02
SA4	0.00	MA4	0.00	LA4	0.00	Myelocyte	0.07
SA5	0.00	MA5	0.02	LA5	0.00	PMN.	26.58
Total SA	0.85	Total MA	0.90	Total LA	1.33	PME.	1.70
SB0	0.83	MB0	0.00	LB0	0.00		
SB1	32.42	MB1	0.50	LB1	0.22		
SB2	0.20	MB2	0.07	LB2	0.10		
SB3	0.97	MB3	0.63	LB3	0.80		
SB4	0.23	MB4	0.62	LB4	0.65		
SB5	0.23	MB5	0.52	LB5	0.88		
Total SB	34.88	Total MB	2.34	Total LB	2.65		
SC1	17.35	MC1	0.87	LC1	0.43		
SC2	1.62	MC2	0.27	LC2	0.10		
SC3	3.40	MC3	0.30	LC3	0.12		
SC4	0.16	MC4	0.13	LC4	0.17		
SC5	0.58	MC5	0.33	LC5	0.35		
Total SC	23.11	Total MC	1.90	Total LC	1.17		

peritoneally with approximately 10,000 parasites. Daily counts were made of parasites beginning the 1st day, and of white blood cells beginning the 2nd day, after inoculation. Table III shows the number of parasites in the blood of these animals from the time of injection to the time of death.

From this table it will be seen that the animals dying early happen to be smaller and the number of parasites in their blood shows a rapid rise reaching its peak with the death of the animal; the animals that survived longer, on the other hand, happen to be larger and the number of parasites shows a gradual rise, then a rapid fall, and finally

TABLE II

The Red Blood Cell Counts and Hemoglobin Values of 10 Rats Before, and 7 Days After, Intraperitoneal Injection of Approximately 10,000 Parasites

Rat No.	Before infection		On the 7th day of infection	
	Red blood cells per cmm.	Hemoglobin	Red blood cells per cmm.	Hemoglobin
		per cent [1]		*per cent*
102	10,790,000	100	5,730,000	65
103	9,770,000	88	7,010,000	73
104	9,410,000	92	6,430,000	64
105	11,110,000	100	7,770,000	78
106	9,790,000	89	7,360,000	63
107	10,620,000	100	7,360,000	65
108	10,030,000	94	6,870,000	70
109	9,730,000	88	7,170,000	70
110	10,140,000	91	7,140,000	73
111	9,300,000	92	7,940,000	78
Average	10,069,000	93.4	7,078,000	69.9

1. 100 per cent = 17 gm. of hemoglobin.

TABLE III

Daily Counts of Parasites in the Blood of 20 Infected White Rats

Group *	Rat No.	Body wt.	Survival period	Day after inoculation								
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th
				Number of parasites in thousands per cmm.								
		gm.	*days*									
A	145	234	5	0	+	6	154	500	..	..	..	..
A	140	251	5	0	0	5	97	475	..	..	..	..
A	143	342	5	0	+	5	155	467	..	..	..	..
A	142	211	6	0	+	7	231	443	430	..	..	..
A	144	304	6	0	0	3	56	385	***	..	..	..
A	141	357	6	0	0	2	101	316	348	..	..	..
A	135	350	6	0	+	3	47	205	526	..	..	..
A	139	307	6	0	0	6	106	290	319	..	..	..
**	136	360	7	0	0	2	62	262	364	392	..	..
**	150	407	7	0	+	4	125	267	352	338	..	..
B	131	440	9	0	+	2	46	116	3	10	195	***
B	147	379	9	0	0	5	91	79	2	20	252	***
B	146	392	9	0	+	5	55	56	1	6	213	587
B	149	335	9	0	+	+	39	99	1	7	194	556
B	133	377	9	0	+	2	56	+	2	6	136	410
B	148	357	9	0	+	3	52	+	1	17	192	560
B	137	320	9	0	+	5	13	0	3	12	164	513
B	134	392	9	0	+	6	60	4	+	14	96	290
B	138	315	9	0	0	2	21	0	1	9	97	327
B	132	430	9	0	+	7	34	155	4	8	300	524

* See text.
** Not included in either Group A or Group B.
*** Animal died before counts could be made.
\+ = less than 1000 per cmm.

TABLE IV

Average Percentage Values of the White Blood Cells Obtained from the Counts Made on 8 Rats (Group A) During Infection, with their Normal Counts as Control

Code name	Normal *	Day after inoculation				
		2nd	3rd	4th	5th	6th**
SA0	0.08	0	0	0.25	2.5	0
SA1	1.25	1.5	2.0	1.0	5.5	3.5
SA3	0	0	0	0	0.25	0
Total SA	1.33	1.5	2.0	1.25	8.25	3.5
SB0	0.79	1.25	2.0	0.25	1.25	0.5
SB1	33.33	25.5	19.5	24.5	21.0	27.0
SB2	0.13	0.5	0	0	0.25	0
SB3	0.79	0	0.75	0.25	0	0
SB4	0.17	0	0	0	0	0
SB5	0.29	0.25	0.25	0	0	0
Total SB	35.5	27.5	22.5	25.0	22.5	27.5
SC1	15.49	18.5	12.0	6.75	7.25	11.0
SC2	2.04	1.75	0.75	0.25	1.0	2.0
SC3	3.71	2.0	1.5	0.25	1.0	1.5
SC4	0.21	0.25	0	0	0	0
SC5	0.54	0.25	0.5	0.5	0	0
Total SC	21.99	22.75	14.75	7.75	9.25	14.5
MA0	0.12	0.25	0	0.75	2.5	0
MA1	0.46	0.25	2.0	0.5	3.75	2.0
MA2	0.04	0	0	0	0	0.5
MA3	0.04	0	0	0	0	0.5
MA5	0.04	0	0	0	0	0.5
Total MA	0.7	0.5	2.0	1.25	6.25	3.5
MB1	0.58	0.75	0.25	0.5	1.5	2.0
MB2	0.04	0	0	0	0	0.5
MB3	0.50	0.25	1.0	0.25	1.0	0.5
MB4	0.62	0	0.25	0	0	0
MB5	0.38	0.25	1.5	0.75	1.0	1.0
Total MB	2.12	1.25	3.0	1.5	3.5	4.0
MC1	1.08	3.0	1.75	1.25	0	0.5
MC2	0.25	0.25	0.25	0	0.5	0
MC3	0.33	0.5	0	0	0	0
MC4	0.21	0	0.25	0.25	0	0
MC5	0.5	1.0	0.5	0	1.0	0
Total MC	2.37	4.75	2.75	1.5	1.5	0.5

TABLE IV (*Continued*)

Code name	Normal *	Day after inoculation				
		2nd	3rd	4th	5th	6th**
LA0	0.08	0.25	0.25	1.0	2.0	1.0
LA1	0.58	0.25	1.0	1.75	2.75	1.0
LA2	0.04	0.25	0	0	0	0
LA3	0.04	0	0.25	0	0.25	0.5
LA5	0	0	0.25	0	0	0
Total LA	0.74	0.75	1.75	2.75	5.0	2.5
LB1	0.21	0.25	0	1.25	2.0	0
LB2	0.08	0	0	0.75	0.75	0
LB3	0.79	0.25	1.5	0.25	1.75	0
LB4	0.58	0.5	0.75	0	1.25	2.0
LB5	0.75	1.0	1.5	0.75	5.75	3.0
Total LB	2.41	2.0	3.75	3.0	11.5	5.0
LC1	0.54	1.75	1.5	1.0	0.75	1.5
LC2	0.08	0.25	0.25	0.25	0	0
LC3	0.13	0.25	0.25	0	0.25	0.5
LC4	0.17	0.5	0	0	0	0
LC5	0.41	1.25	1.0	1.0	1.0	1.5
Total LC	1.33	4.0	3.0	2.25	2.0	3.5
Deg.	0.04	0.5	0	2.25	0.5	1.5
Mono.	1.87	2.75	3.5	1.25	3.25	6.0
Plasma	0	0	0	0	0	0
Clas.	0.04	0	0	0	0.5	1.0
Myelo.	0.04	0	0.25	0	0	0
PMN	27.83	30.5	37.25	47.5	24.0	25.0
PME	1.63	1.25	3.5	2.75	2.0	2.0
Average total WBC per cmm.	8110	8150	7790	4825	6710	8970

* These values are incorporated in Table I.
** Counts from only 4 animals.

a sharp rise shortly before death. The survival period of the animals and the rate of increase of parasites vary, therefore, according to the intensity of infection, influenced by the size of the animals used. But as this finding has no direct bearing on the problem on hand no further discussion will be made.

In view of the wide variation in the survival period of these animals it is apparent that an average of the white counts of all of them

will be of very little value. These counts are therefore divided into two groups. In Group A are placed the counts of 8 rats dying on the 5th or 6th day of infection, and in Group B those of 10 rats dying on the 8th or 9th day. The counts of 2 animals (Nos. 136 and 150) which died on the 7th day cannot be conveniently placed in either group and are therefore discarded.

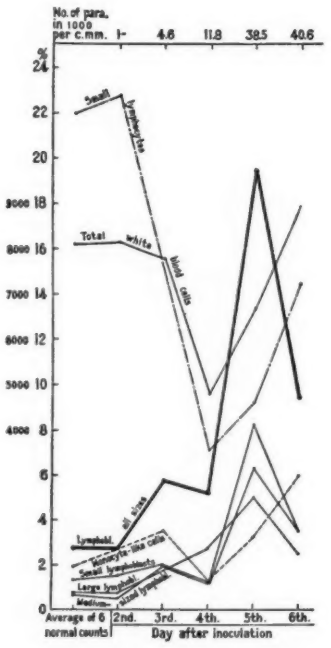

CHART 1

A graphic representation of the important data in Table IV. The differential counts are recorded in percentages; the white blood cell counts in total numbers per cmm.

The average values of the counts in Group A are shown in Table IV and Chart 1; those in Group B in Table V and Chart 2.

The important points revealed by Table IV are as follows. (1) A transient leukopenia, most pronounced on the 4th day of infection, due largely to the reduction in number of small lymphocytes. (2) A marked increase in number of lymphoblasts of all sizes and a similar increase of the medium sized and large immature lympho-

TABLE V

Average Percentage Values of the White Blood Cells Obtained from the Counts of 10 Rats (Group B) during Infection, with their Normal Counts as Control

Code name	Normal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
SA0	0	0	0	0	1.4	0.4	0.2	0.4	0.25
SA1	0.63	1.8	1.0	2.6	6.0	1.8	3.2	0.4	2.0
SA3	0.03	0	0	0	0.6	0	0.2	0	0
Total SA	0.66	1.8	1.0	2.6	8.0	2.2	3.6	0.8	2.25
SB0	0.83	3.2	1.2	0.4	1.8	0.8	1.4	0.8	0.5
SB1	33.13	31.6	25.8	21.6	28.4	29.4	31.0	26.6	22.5
SB2	0.27	0	0	0.2	0.4	0	0.2	0.4	0
SB3	1.2	1.0	1.8	0	0.2	0.2	1.6	0.6	0.25
SB4	0.17	0.2	0.4	0	0	0	0.2	0	0.25
SB5	0.2	0.2	1.0	0.2	0	0.2	0.6	0.6	0
Total SB	35.8	36.2	30.2	22.4	30.8	30.6	35.0	29.0	23.5
SC1	19.03	16.2	11.6	9.6	11.8	21.2	17.8	13.2	8.0
SC2	1.3	2.0	0	1.0	0.6	1.2	2.0	1.0	0.75
SC3	3.23	2.0	2.4	0.2	0.2	1.4	3.2	3.0	4.5
SC4	0.17	0	0.2	0	0	0.2	0	0	0.25
SC5	0.47	0.4	0.6	0.2	0	0	0.6	0.6	0
Total SC	24.2	20.6	14.8	11.0	12.6	24.0	23.6	17.8	13.5
MA0	0.1	0	0	0	1.8	0.4	0	0.6	1.0
MA1	0.77	0	0.6	0.8	4.6	1.6	1.4	1.	1.25
MA2	0	0	0	0	0.2	0	0.2	0	0
MA3	0	0	0	0.2	0	0	0	0.2	0
MA5	0	0	0.2	0	0	0.2	0.6	0	0.25
Total MA	0.87	0	0.8	1.0	6.6	2.2	2.2	1.8	2.5
MB1	0.33	0.6	0.6	0.8	0.8	2.0	0.6	1.6	0.25
MB2	0.07	0.2	0	0	0.2	0.2	0.2	0	0.25
MB3	0.8	1.2	0.8	0.6	0.2	0.6	1.0	1.6	0.25
MB4	0.53	1.4	0.2	0	0.4	0.2	0.6	1.0	0.25
MB5	0.67	1.0	1.0	0.4	0.6	0.6	1.6	1.2	1.75
Total MB	2.4	4.4	2.6	1.8	2.2	3.6	4.0	5.4	2.75
MC1	0.77	2.2	0.2	0.2	0.2	1.6	2.6	2.0	0.5
MC2	0.2	0.4	0	0	0	0.6	0.6	0	0.25
MC3	0.2	0.6	0.6	0.2	0	0	0.4	0.6	0.25
MC4	0.1	0.2	0	0	0	0	0.4	0.4	0.25
MC5	0.23	0.2	0.6	0	0	0.2	0.2	0	0.75
Total MC	1.5	3.6	1.4	0.4	0.2	2.4	4.2	3.0	2.0

TABLE V (*Continued*)

Code name	Normal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
LA0	0.2	0.2	0	1.2	2.4	0.8	0.4	0.4	0.75
LA1	1.23	0.8	1.4	1.4	2.8	1.4	1.0	1.0	1.0
LA2	0.03	0	0	0.2	0	0	0	0	0
LA3	0	0.2	0.4	0.2	0.4	0.4	0.4	0	0
LA5	0	0	0.2	0	0.4	0	0	0.4	0.25
Total LA	1.46	1.2	2.0	3.0	6.0	2.6	1.8	1.8	2.0
LB1	0.2	0.2	0.2	0.8	1.0	0.8	0.2	0.6	0.5
LB2	0.13	0	0.2	0.2	0.2	0	0.4	0.2	0.25
LB3	0.83	0.2	1.4	1.4	2.4	0.6	0	1.0	0.75
LB4	0.53	0	1.2	0.2	0	1.4	0.4	0.2	1.25
LB5	0.83	0.4	1.6	1.2	3.0	1.0	1.4	2.4	3.5
Total LB	2.52	0.8	4.6	3.8	6.6	3.8	2.4	4.4	6.25
LC1	0.43	0.6	0.2	0	0.4	1.0	0.4	1.2	1.75
LC2	0.07	0	0	0.2	0	0.2	0.4	0	0.25
LC3	0.07	0.2	0	0	0	0	0.2	0.2	0.25
LC4	0.17	0	0	0	0	0	0	0.2	0
LC5	0.2	0.2	0.8	0.4	0.8	1.6	0.8	0.4	1.0
Total LC	0.94	1.0	1.0	0.6	1.2	2.8	1.8	2.0	3.25
Deg.	0.02	3.0	1.2	5.0	7.2	7.6	2.4	2.0	1.25
Mono.	2.30	1.6	1.8	2.4	3.2	5.8	3.6	4.4	5.5
Plasma	0	0	0	0	0	0	0	0	0.25
Clas.	0	0	0	0	0.2	0	0.2	0	0
Myelo.	0.47	0	0	0	0	0	0	0	0
PMN	25.33	23.8	35.8	44.0	14.2	11.0	12.4	24.2	29.5
PME	1.3	2.0	2.8	2.0	1.0	1.4	2.8	3.4	5.5
Average total WBC per cmm.	8610	8140	6950	5730	8100	8450	9170	7180	6680

* These values are incorporated in Table I.
** Counts from only 8 animals.

number and to show increased physical activity. It seems desirable, therefore, to classify these subvarieties simply into two main groups, the typical and the atypical. To the former belong cell Types 0, 1 and 2 and to the latter 3, 4 and 5.

In regard to the cells having the appearance of monocytes we are unable to say whether they are true monocytes or if they merely represent another variation of the atypical lymphoid cells. It is quite definite, however, that they can be traced easily to the atypical

cytes (especially LB5) on the 5th day. (3) An increase in number of monocyte-like cells at the end of infection. These points are graphically shown in Chart 1.

Table V and Chart 2 show the following additional features. (1) The number of small lymphocytes, which shows a sharp fall on the 4th day of infection, as in Table IV, rises to its normal level on the 6th day, but again falls to a lower level at the time of death. (2) The number of lymphoblasts, after a rise on the 5th day, later also falls, but not below the normal level. (3) A few plasma cells appear in the blood at the end of infection.

It was noted that in the different types of lymphoid cells and in the monocyte-like cells the neutral red bodies tend to increase both [illegible] in the latter part of infection. These cells (Figs.

ERRATUM

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liferative power of what are [illegible]
cytes of the blood.

At the beginning of this report an elaborate classification of the non-granular white blood cells of the rat was given. Examination of the data in this experiment indicates, however, that although it is necessary to separate the lymphoid cells according to their age and size, the classification of each type into six subvarieties (Types 0, 1, 2, 3, 4 and 5) is not essential. Attention is called to the fact that these subvarieties actually form a continuous series of cells and that the morphology of some of them is by no means constant, but is apt to change from one type to another (Figs. 79, 80). On the other hand, during infection some of these subvarieties, especially Type 5 of the medium sized and the large cells, tend to increase in

will be of very little value. These counts are therefore divided into two groups. In Group A are placed the counts of 8 rats dying on the 5th or 6th day of infection, and in Group B those of 10 rats dying on the 8th or 9th day. The counts of 2 animals (Nos. 136 and 150) which died on the 7th day cannot be conveniently placed in either group and are therefore discarded.

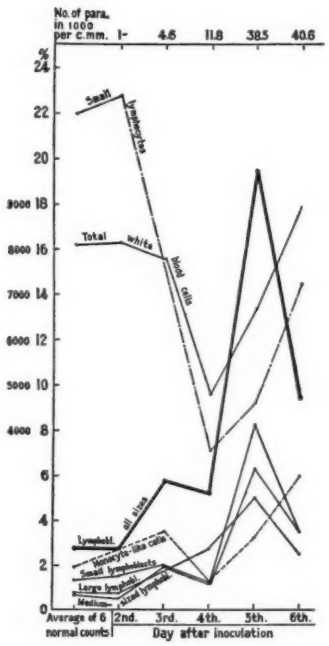

CHART 1

A graphic representation of the important data in Table IV. The differential counts are recorded in percentages; the white blood cell counts in total numbers per cmm.

The average values of the counts in Group A are shown in Table IV and Chart 1; those in Group B in Table V and Chart 2.

The important points revealed by Table IV are as follows. (1) A transient leukopenia, most pronounced on the 4th day of infection, due largely to the reduction in number of small lymphocytes. (2) A marked increase in number of lymphoblasts of all sizes and a similar increase of the medium sized and large immature lympho-

TABLE V

Average Percentage Values of the White Blood Cells Obtained from the Counts of 10 Rats (Group B) during Infection, with their Normal Counts as Control

Code name	Normal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
SA0	0	0	0	0	1.4	0.4	0.2	0.4	0.25
SA1	0.63	1.8	1.0	2.6	6.0	1.8	3.2	0.4	2.0
SA3	0.03	0	0	0	0.6	0	0.2	0	0
Total SA	0.66	1.8	1.0	2.6	8.0	2.2	3.6	0.8	2.25
SB0	0.83	3.2	1.2	0.4	1.8	0.8	1.4	0.8	0.5
SB1	33.13	31.6	25.8	21.6	28.4	29.4	31.0	26.6	22.5
SB2	0.27	0	0	0.2	0.4	0	0.2	0.4	0
SB3	1.2	1.0	1.8	0	0.2	0.2	1.6	0.6	0.25
SB4	0.17	0.2	0.4	0	0	0	0.2	0	0.25
SB5	0.2	0.2	1.0	0.2	0	0.2	0.6	0.6	0
Total SB	35.8	36.2	30.2	22.4	30.8	30.6	35.0	29.0	23.5
SC1	19.03	16.2	11.6	9.6	11.8	21.2	17.8	13.2	8.0
SC2	1.3	2.0	0	1.0	0.6	1.2	2.0	1.0	0.75
SC3	3.23	2.0	2.4	0.2	0.2	1.4	3.2	3.0	4.5
SC4	0.17	0	0.2	0	0	0.2	0	0	0.25
SC5	0.47	0.4	0.6	0.2	0	0	0.6	0.6	0
Total SC	24.2	20.6	14.8	11.0	12.6	24.0	23.6	17.8	13.5
MA0	0.1	0	0	0	1.8	0.4	0	0.6	1.0
MA1	0.77	0	0.6	0.8	4.6	1.6	1.4	1.	1.25
MA2	0	0	0	0	0.2	0	0.2	0	0
MA3	0	0	0	0.2	0	0	0	0.2	0
MA5	0	0	0.2	0	0	0.2	0.6	0	0.25
Total MA	0.87	0	0.8	1.0	6.6	2.2	2.2	1.8	2.5
MB1	0.33	0.6	0.6	0.8	0.8	2.0	0.6	1.6	0.25
MB2	0.07	0.2	0	0	0.2	0.2	0.2	0	0.25
MB3	0.8	1.2	0.8	0.6	0.2	0.6	1.0	1.6	0.25
MB4	0.53	1.4	0.2	0	0.4	0.2	0.6	1.0	0.25
MB5	0.67	1.0	1.0	0.4	0.6	0.6	1.6	1.2	1.75
Total MB	2.4	4.4	2.6	1.8	2.2	3.6	4.0	5.4	2.75
MC1	0.77	2.2	0.2	0.2	0.2	1.6	2.6	2.0	0.5
MC2	0.2	0.4	0	0	0	0.6	0.6	0	0.25
MC3	0.2	0.6	0.6	0.2	0	0	0.4	0.6	0.25
MC4	0.1	0.2	0	0	0	0	0.4	0.4	0.25
MC5	0.23	0.2	0.6	0	0	0.2	0.2	0	0.75
Total MC	1.5	3.6	1.4	0.4	0.2	2.4	4.2	3.0	2.0

TABLE V (*Continued*)

Code name	Normal*	Day after inoculation							
		2nd	3rd	4th	5th	6th	7th	8th	9th**
LA0	0.2	0.2	0	1.2	2.4	0.8	0.4	0.4	0.75
LA1	1.23	0.8	1.4	1.4	2.8	1.4	1.0	1.0	1.0
LA2	0.03	0	0	0.2	0	0	0	0	0
LA3	0	0.2	0.4	0.2	0.4	0.4	0.4	0	0
LA5	0	0	0.2	0	0.4	0	0	0.4	0.25
Total LA	1.46	1.2	2.0	3.0	6.0	2.6	1.8	1.8	2.0
LB1	0.2	0.2	0.2	0.8	1.0	0.8	0.2	0.6	0.5
LB2	0.13	0	0.2	0.2	0.2	0	0.4	0.2	0.25
LB3	0.83	0.2	1.4	1.4	2.4	0.6	0	1.0	0.75
LB4	0.53	0	1.2	0.2	0	1.4	0.4	0.2	1.25
LB5	0.83	0.4	1.6	1.2	3.0	1.0	1.4	2.4	3.5
Total LB	2.52	0.8	4.6	3.8	6.6	3.8	2.4	4.4	6.25
LC1	0.43	0.6	0.2	0	0.4	1.0	0.4	1.2	1.75
LC2	0.07	0	0	0.2	0	0.2	0.4	0	0.25
LC3	0.07	0.2	0	0	0	0	0.2	0.2	0.25
LC4	0.17	0	0	0	0	0	0	0.2	0
LC5	0.2	0.2	0.8	0.4	0.8	1.6	0.8	0.4	1.0
Total LC	0.94	1.0	1.0	0.6	1.2	2.8	1.8	2.0	3.25
Deg.	0.02	3.0	1.2	5.0	7.2	7.6	2.4	2.0	1.25
Mono.	2.30	1.6	1.8	2.4	3.2	5.8	3.6	4.4	5.5
Plasma	0	0	0	0	0	0	0	0	0.25
Clas.	0	0	0	0	0.2	0	0.2	0	0
Myelo.	0.47	0	0	0	0	0	0	0	0
PMN	25.33	23.8	35.8	44.0	14.2	11.0	12.4	24.2	29.5
PME	1.3	2.0	2.8	2.0	1.0	1.4	2.8	3.4	5.5
Average total WBC per cmm.	8610	8140	6950	5730	8100	8450	9170	7180	6680

* These values are incorporated in Table I.
** Counts from only 8 animals.

number and to show increased physical activity. It seems desirable, therefore, to classify these subvarieties simply into two main groups, the typical and the atypical. To the former belong cell Types 0, 1 and 2 and to the latter 3, 4 and 5.

In regard to the cells having the appearance of monocytes we are unable to say whether they are true monocytes or if they merely represent another variation of the atypical lymphoid cells. It is quite definite, however, that they can be traced easily to the atypical

ERRATUM

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cytes (especially LB_5) on the 5th day. (3) An increase in number of monocyte-like cells at the end of infection. These points are graphically shown in Chart 1.

Table V and Chart 2 show the following additional features. (1) The number of small lymphocytes, which shows a sharp fall on the 4th day of infection, as in Table IV, rises to its normal level on the 6th day, but again falls to a lower level at the time of death. (2) The number of lymphoblasts, after a rise on the 5th day, later also falls, but not below the normal level. (3) A few plasma cells appear in the blood at the end of infection.

It was noted that in the different types of lymphoid cells and in the monocyte-like cells the neutral red bodies tend to increase both in number and in size in the latter part of infection. These cells (Figs. 29, 32, 33, 79–86), which are usually large, are often very active. The shape of these cells and their nuclei changes rapidly and the enlarged neutral red bodies are constantly in motion, so that the same cell may appear quite differently at different times (Figs. 79, 80).

It may also be mentioned that the lymphoblasts, usually the large variety, are occasionally seen undergoing mitosis (Fig. 65).

Discussion

In connection with the results obtained in this and the previous studies,[1,2] three problems are to be considered: the classification of the non-granular white blood cells of the rat, the relation between the tissue lymphoid cells and the blood lymphoid cells, and the proliferative power of what are generally known as the small lymphocytes of the blood.

At the beginning of this report an elaborate classification of the non-granular white blood cells of the rat was given. Examination of the data in this experiment indicates, however, that although it is necessary to separate the lymphoid cells according to their age and size, the classification of each type into six subvarieties (Types 0, 1, 2, 3, 4 and 5) is not essential. Attention is called to the fact that these subvarieties actually form a continuous series of cells and that the morphology of some of them is by no means constant, but is apt to change from one type to another (Figs. 79, 80). On the other hand, during infection some of these subvarieties, especially Type 5 of the medium sized and the large cells, tend to increase in

lymphoid cells and, like the latter, are also increased in number in this infection.

We may now present our classification of the non-granular white blood cells of the rat as follows:

1. Lymphoblasts	small,	medium sized,	large.
2. Atypical lymphoblasts	"	"	"
3. Immature lymphocytes	"	"	"
4. Atypical immature lymphocytes	"	"	"
5. Lymphocytes	"	"	"
6. Atypical lymphocytes	"	"	"
7. Degenerated lymphoid cells			
8. Monocyte-like cells			
9. Plasma cells			
10. Clasmatocytes			

In regard to the relation between the lymphoid cells of the tissue and the blood it is first of all important to know whether the increased number of lymphoblasts in the blood during infection is due to an increased emigration from the spleen and lymph nodes, or whether it is due to direct proliferation of the circulating lymphoblasts. It is probable that lymphoblasts without neutral red bodies are emigrated cells, since most of the lymphoblasts in the spleen and lymph nodes are of this type. But emigration of lymphoblasts does not seem to take place readily for, although many large lymphoblasts appear in the spleen as early as 48 hours after the animals become infected,[1] the blood does not show any increase of such cells until 2 or 3 days later, when many parasites appear in the circulation. Furthermore, when the medium sized lymphoblasts are formed in great numbers in the lymphoid organs in the latter part of infection there is no corresponding increase of such cells in the blood. On the other hand, the repeated finding of mitoses among the circulating lymphoblasts is a further evidence of the local origin of some of these cells.

In view of the great severity of the infection and the presence in the blood stream of tremendous numbers of parasites toward the end of the disease the reduction in number of blood lymphoblasts at this time, as shown in Table V, needs to be explained. It will be recalled in this connection that at this stage of the disease sections of these organs, especially the spleen, also showed a definite reduction in number of lymphoblasts. We are inclined to think that it is due either to excessive cell destruction, or to exhaustion of the prolifera-

tive power of the lymphoblasts in the presence of an overwhelming infection.

The presence of a higher percentage of small lymphocytes in the blood than in the lymphoid organs suggests either one or both of the following two possibilities: that the small lymphocytes of the organs

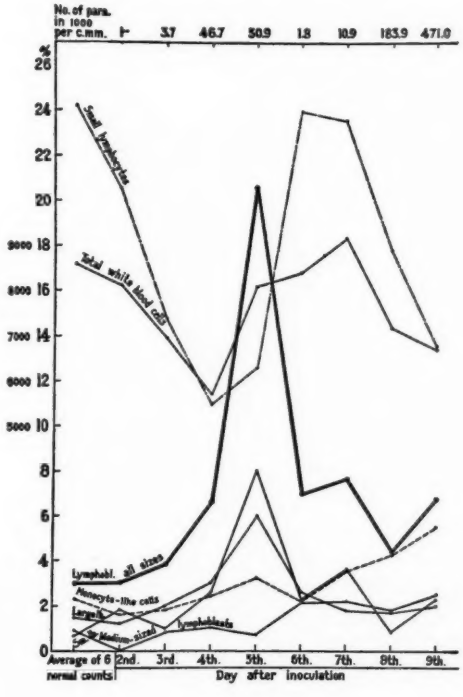


CHART 2

A graphic representation of the important data in Table V. The differential counts are recorded in percentages; the white blood cells in total numbers per cmm.

emigrate more readily than the immature lymphocytes, or that the latter cells maturate more quickly in circulation than in the tissue. The marked reduction in small lymphocytes, which is largely responsible for the initial leukopenia in the infected animals, is interesting and can probably be explained by the fact that since they are older they are more susceptible to injury than the others.

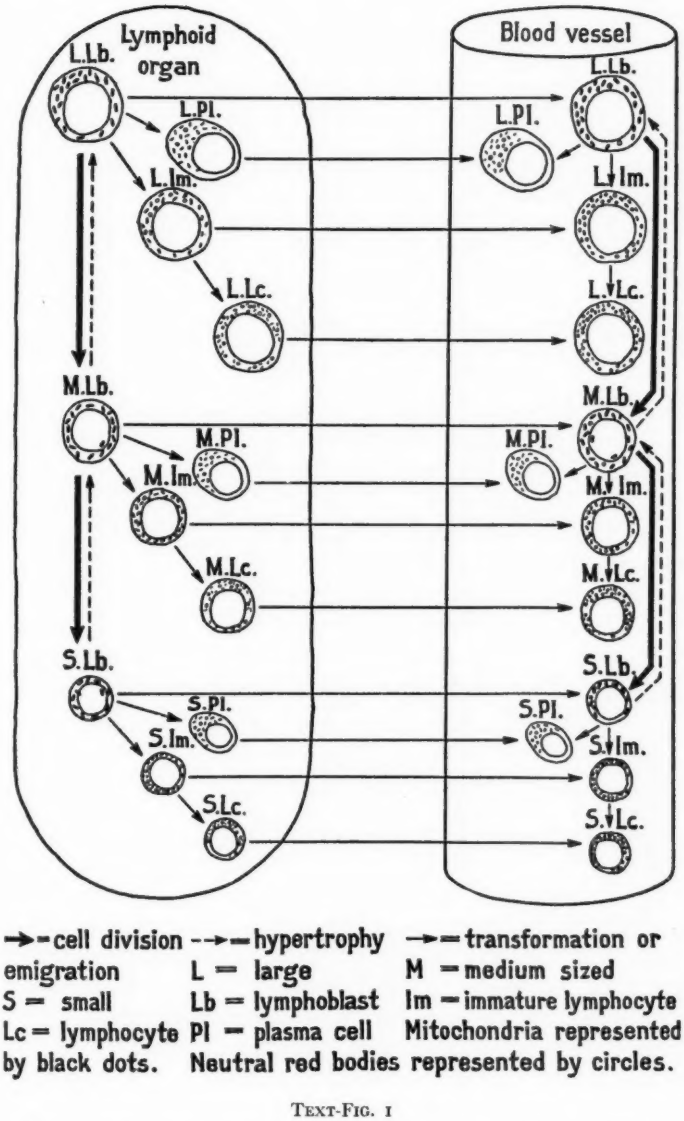
Although many plasma cells are produced in the tissues by the transformation of the lymphoblasts, very few such cells are seen in the circulation. Like the lymphoblasts they do not readily pass into the blood stream. On the other hand, it is still possible that some of the circulating lymphoblasts may be transformed into plasma cells, since they are morphologically identical with the tissue lymphoblasts, whose ability for such a transformation seems to be quite definite.

Text-figure 1 represents the relation between the different types of tissue and blood lymphoid cells.

Although what is generally called the small lymphocyte of the blood has been studied by many investigators, its potentialities for hypertrophy, proliferation and transformation are still a subject of dispute. Representing probably the more general view is Naegeli,[3] who considers that all the small lymphoid cells of the blood are true mature lymphocytes, incapable of further growth or transformation. He believes, however, that the small lymphoid cells of the lymphoid organs are young cells, capable of forming large lymphoblasts by hypertrophy. The blood lymphocytes, according to him, are derived from these lymphoblasts.

Cunningham, Sabin, and Doan [4] also believe that the so-called small lymphocytes of the blood are more mature than the other cells of the lymphoid series. But as mitoses have been observed in these cells, they placed them in their schema on the same level with myelocytes, and not with polymorphonuclear leukocytes of the granular series. They differ from Naegeli, who holds that the size of the cell has nothing to do with its maturity, and look upon the large lymphocytes as being younger than the small lymphocytes. They derive lymphocytes from lymphoblasts and the latter from primitive cells normally present in lymphoid organs.

An entirely different view is expressed by the representatives of the unitarian theory championed especially by Maximow.[5-9] He believes that there is no difference between the lymphocytes of the blood and those of the tissue, and that the small lymphocytes of the blood are, like the larger lymphocytes, potentially capable of unrestricted development, even into cells other than those of the lymphoid series. In culture and in ligated blood vessels he has observed hypertrophy of the small lymphocytes of the blood and their eventual transformation into many other types of cells — monocytes, epithelioid cells, fibroblasts.



TEXT-FIG. 1

A representation of the relation between the different types of tissue and blood lymphoid cells

We are inclined to think that the conflicting views of Naegeli and Maximow are based upon observations made under imperfect experimental conditions. For a satisfactory study of the development of lymphoid cells it is necessary to have not only a state of marked lymphoblastic hyperplasia, which enables the observer to follow closely the rapid changes in the lymphoid cells, but also a technique that makes possible the differentiation of cells which, in fixed smears or tissues, have an identical appearance. Separation of the blood lymphoid cells into lymphoblasts, immature lymphocytes and lymphocytes * offers an easy explanation to the above-mentioned conflicting views. It is apparently the presence of lymphoblasts that has led some to the conclusion that *all* lymphoid cells are primitive cells; while the presence of mature lymphocytes led others to believe *all* blood lymphoid cells to be true lymphocytes, that is, the end cells of the lymphoid series.

Summary

In contrast to the general belief that the lymphoid cells of the peripheral blood of normal animals are end cells, the present study shows that irrespective of their size they are actually composed of lymphoblasts, immature lymphocytes and lymphocytes. In the blood of rats infected with *Trypanosoma brucei* the number of lymphoblasts shows a marked increase on the fifth day of infection, as a result of proliferation of the normally existing, circulating lymphoblasts, and emigration of tissue lymphoblasts from the spleen and lymph nodes. After the 6th day this number decreases, in spite of the increased severity of the disease, probably as a result of increased cell destruction or exhaustion of their proliferative power.

A new classification of the non-granular white blood cells of the rat and a text-figure illustrating the relation between the tissue and blood lymphoid cells are presented in the text.

* Such a separation of the blood lymphoid cells is also possible in normal rabbits and human beings.

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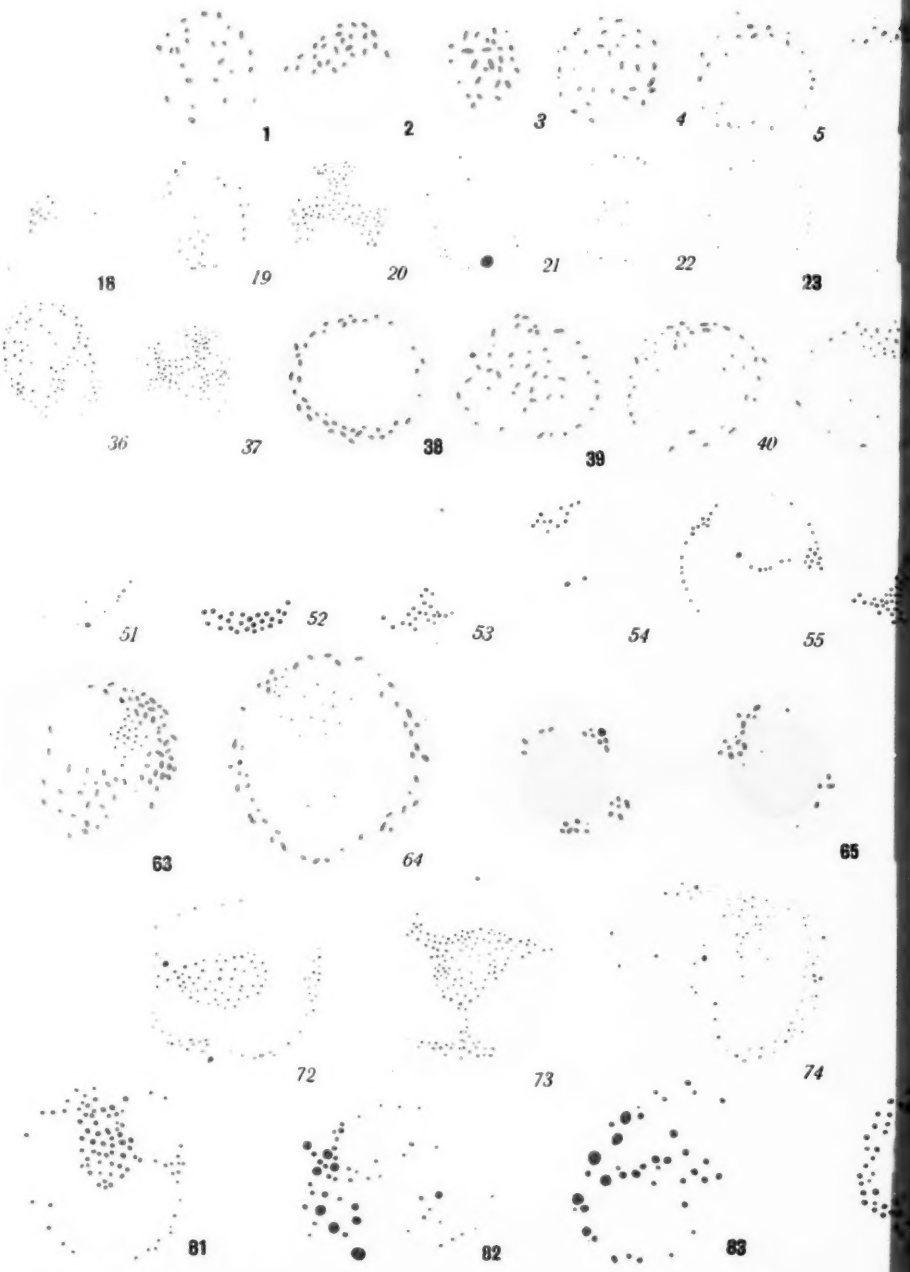
DESCRIPTION OF PLATE

PLATE 42

All the cells illustrated in the colored plate were drawn within 10 minutes after the films were made. They were from the blood obtained by puncturing the saphenous veins of 6 normal and 8 infected rats. Cells from infected animals have their numbers printed in arabic type, those of the normal animals in italics. The size of a red blood cell of the rat is given for comparison.

The following types of cells are illustrated:

Small lymphoblasts — 1–9.
Small immature lymphocytes — 10–19.
Atypical small immature lymphocyte — 20.
Small lymphocytes — 21–30.
(Cell No. 24 underwent degeneration and later appeared as cell No. 25.)
Atypical small lymphocytes — 31–37.
Medium sized lymphoblasts — 38–41.
Medium sized immature lymphocytes — 42–44.
Atypical medium sized immature lymphocytes — 45–48.
Medium sized lymphocytes — 49–54.
Atypical medium sized lymphocytes — 55, 56.
Large lymphoblasts — 57–64.
Large lymphoblast in mitosis — 65.
Large plasma cell — 66.
Large immature lymphocytes — 67–70.
Atypical large immature lymphocytes — 71–75.
Large lymphocytes — 76, 77.
Atypical large lymphocytes — 78–86.
(Cell No. 79 was in active motion; in a few minutes it appeared as cell No. 80.)
Monocyte-like cells — 87–90.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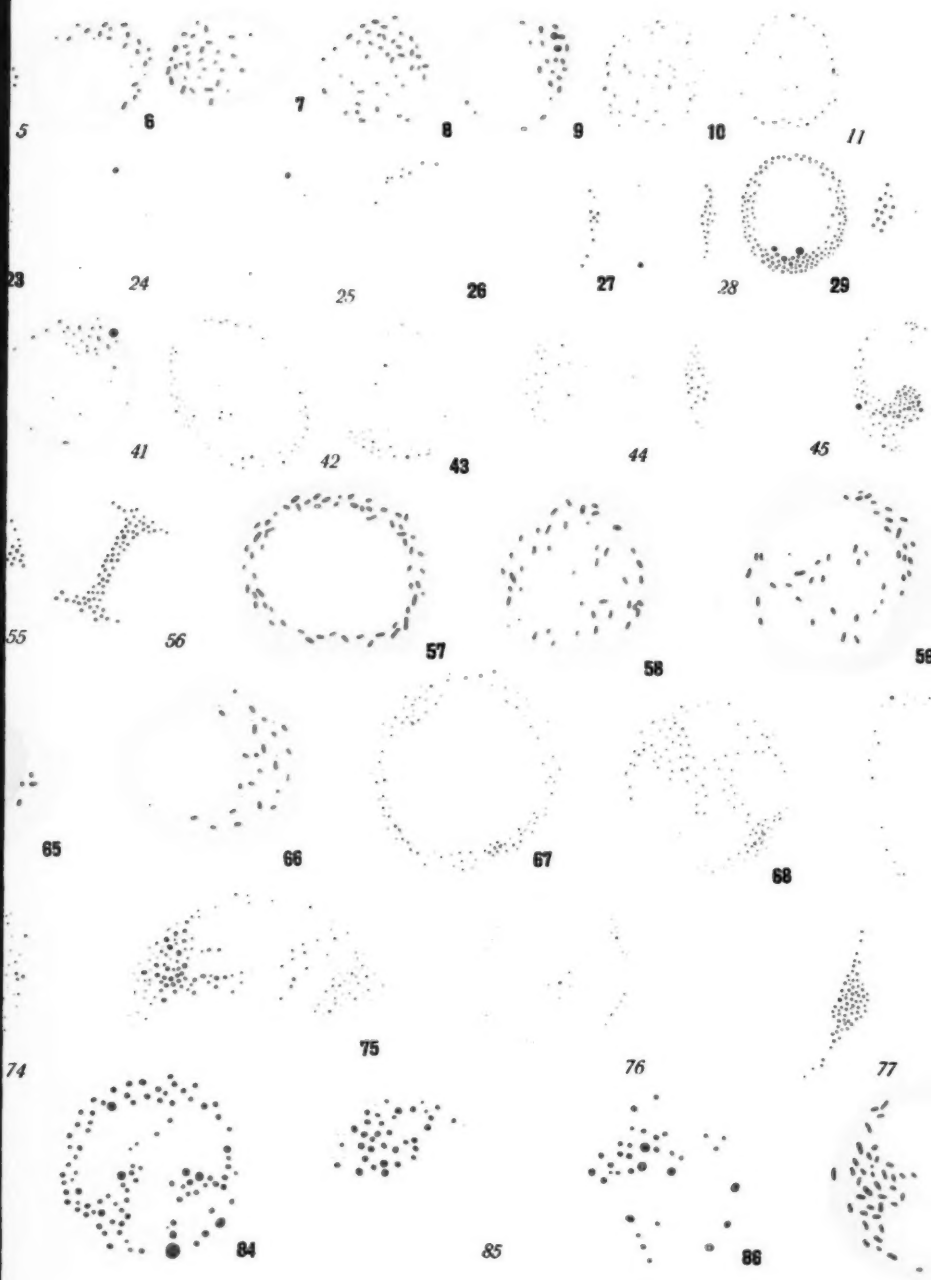
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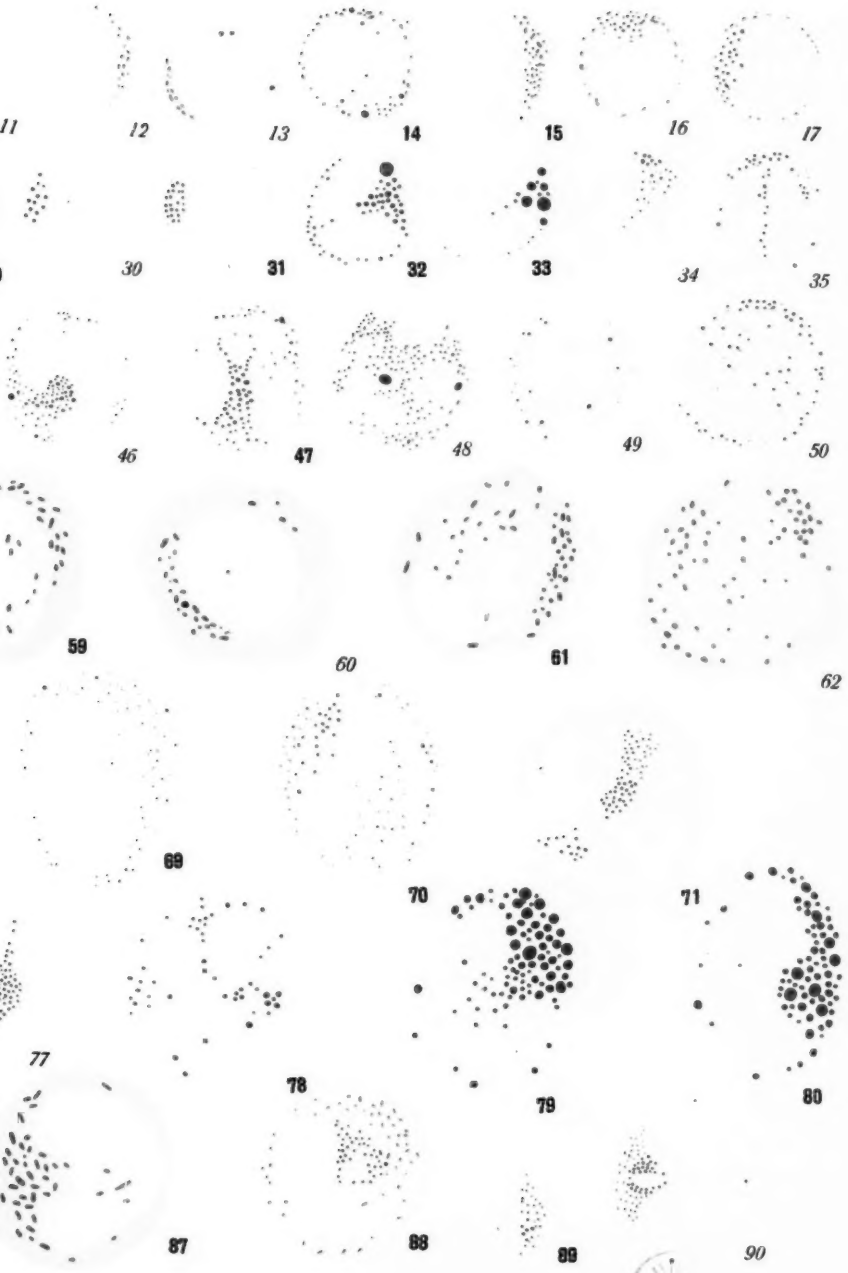

Lymphoid Cells of Peripheral Blood

THE HEART VALVES AND MUSCLE IN EXPERIMENTAL SCURVY WITH SUPERIMPOSED INFECTION *

WITH NOTES ON THE SIMILARITY OF THE LESIONS TO THOSE OF RHEUMATIC FEVER

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In experiments designed primarily to study mesenchymal reactions in scorbutic guinea pigs subjected to infection certain degenerative and proliferative lesions were observed in the heart valves. These observations led to a series of experiments directed to the determination of the effect upon the heart valves and muscle of the following: (1) uncomplicated scurvy, acute and chronic; (2) scurvy combined with infection, and (3) infection alone.

METHOD AND PROCEDURE

Diet: The diet used was a slight modification of one used by Dalldorf.[1] Essentially similar diets have been employed by a number of investigators. The exact composition of the diet used in the experiments is set forth below.

The Basal Diet Employed in Experiments

	Per cent
Baked skimmed milk powder (baked at 110 to 120°C for 2 hours)	30.0
Ground rolled oats and bran — equal parts by volume	56.0
Butter fat	10.0
Dried yeast (Fleischmann's yeast for animals and poultry)	1.5
Cod liver oil (standardized)	1.0
Sodium chloride	1.0
Ferrous lactate	0.5

All ingredients were intimately mixed. The mixed food was kept in the refrigerator. This basal diet and water were given *ad lib.*

The diet lacks only vitamin C. It has proved quite adequate for growth and maintenance when supplemented with orange juice. One control animal has

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been so maintained in excellent condition for 6 months. Orange juice was given as desired in measured amounts by pipette.

The Infecting Agent and Method of Infection

The infecting organism used in the bulk of the experiments here reported was a *beta* hemolytic streptococcus, which is the causative agent of spontaneously occurring cervical lymphadenitis frequently encountered in guinea pigs. The organism is a favorable one in that it is readily available, the infection is easily transmitted and the strains used produce a chronic localized infection that only rarely causes death spontaneously and usually remains confined to regional lymph nodes. Although the streptococci employed were all derived from spontaneous cervical adenitis and were all hemolytic, no attempt was made to use a single strain in the different series of experiments. Infection was transmitted by intracutaneous inoculation of pure cultures of the organism grown on Avery's glucose veal broth. From 0.05 to 1 cc. was inoculated into the skin of the thigh below the groin. A skin pustule develops at the site of inoculation followed shortly by localization, tumefaction, and later suppuration of the regional lymph nodes. Infection in the scorbutic animals in general gave larger lesions which persisted longer and were less well encapsulated and localized.

Care was exercised to secure animals without evidence of spontaneous infection. Infected and non-infected animals were kept in separate rooms.

Pathological Examination

Complete postmortem examinations were performed in the majority of animals. Particular attention was given to the heart examinations and many sections were taken from each heart. In most instances the hearts were incised sufficiently to permit penetration of the fixative. (Zenker's solution was employed in most cases.) The hearts were usually trimmed, after being placed in 80 per cent alcohol, in such a way that the mitral and tricuspid valves would be sectioned simultaneously, and later the aortic and pulmonary valves. It was not possible to do serial sections on the hearts but large numbers of sections were cut. The mitral valve received the greatest attention. In several instances entirely inadequate sections were obtained, due to faults in trimming, embedding or sectioning. In spite of this, sections obtained are considered quite adequate for interpretation. Somewhat more than 5000 sections of hearts were examined.

The Experiments

Four series of experiments were conducted — each series comprising groups of 18 to 30 animals. A broad outline of the groups in each series of experiments is given below, tabulating in brief the material upon which pathological studies were made. Representative protocols of each group, giving detail of procedure, are appended at the end of the paper.

Symbols

Control = basal diet supplemented with adequate amounts of orange juice.
Infection only = animals infected and maintained on basal diet supplemented with adequate amounts of orange juice.
Total scurvy = basal diet without supplement.
Subacute scurvy = basal diet supplemented with inadequate amounts of orange juice.
Chronic scurvy = basal diet supplemented with inadequate amounts of orange juice. Animals maintained longer than subacute group. In the present series of experiments the vitamin C deficiencies were rather severe.

TABLE I

Experiment I

No. of animals	Status	Series No.
2	Control	65–66
4	Total scurvy	47–48–49–50
5	Total scurvy plus infection (*beta* streptococcus)	53–54–55–57–58
3	Infection only (*beta* streptococcus)	59–61–62
1	Infection plus scurvy — late (*beta* streptococcus)	60
1	Infection only — subcutaneous (*B. aertrycke*)	63
1	Infection with terminal scurvy (*B. aertrycke*)	64
2	Total scurvy plus infection — subcutaneous (*B. aertrycke*)	51–52
2	Infection only — intravenous (*B. aertrycke*)	69–70
2	Subacute scurvy plus infection — intravenous (*B. aertrycke*)	67–68

TABLE II

Experiment II

No. of animals	Status	Series No.
4	Control	75–78–79–80
6	Infection only (*beta* streptococcus)	71–72–73–74–76–77
3	Total scurvy	86–87–90
6	Total scurvy plus infection (*beta streptococcus*)	81–83–84–85–88–89

Note: Animals in this series were infected 8 days after being placed on the basal diet. The animals of this group were small, ranging from 185 to 270 gm. Lesions were less well defined in this group because of the short duration of life of very young pigs with scurvy. Animals averaging about 350 gm. are more satisfactory. Animals 71 and 84 received nucleotide K–96 to study blood response.

TABLE III

Experiment III

No. of animals	Status	Series No.
2	Control	104–106
4	Infection only (*beta* streptococcus)	101–102–103–104–105
2	Subacute scurvy only	109–110
3	Subacute scurvy plus infection (*beta* streptococcus)	107–108–111
1	Chronic scurvy — terminal total scurvy	114
1	Chronic scurvy only	117
2	Chronic scurvy plus infection (*beta* streptococcus)	115–118
2	Chronic scurvy plus infection — terminal total scurvy (*beta* streptococcus)	119–120

Note: In this experiment the infected animals were inoculated at the same time with 0.05 cc. of a 28 hour broth culture. This organism produced minimal infections and in some instances none. The entire group was reinoculated 6 days later in the opposite thigh with 0.1 cc. of another hemolytic streptococcus from a different source.

TABLE IV

Experiment IV

No. of animals	Status	Series No.
2	Control	130–131
4	Chronic scurvy only	146–147–148–149
7	Infection only (*beta* streptococcus)	132–133–134–135–136–137–138
4	Chronic scurvy plus infection (*beta* streptococcus) adequate survival	150–151–152–156
3	Chronic scurvy plus infection (*beta* streptococcus) short survival	153–154–155
2	Infection only (*beta* streptococcus) reinoculation	139–140
2	Chronic scurvy plus infection (*beta* streptococcus) reinoculation	157–159
4	Infection (*beta* streptococcus) followed by subacute scurvy	142–143–144–145

Note: More detailed data on precise treatment of individual animals may be had by referring to appended protocols. The chronic scurvy animals subjected to superimposed infection that survived infection for longer periods showed in general more definite pathological pictures. We feel that there is no significant difference in the reinoculated groups. The local reactions to reinoculation were mild, of short duration and showed no evidence of spread.

For the sake of clarity the findings in the four series will be considered as a whole. The results in each series of animals are consistent enough in principle to

warrant this method of consideration. In the heart examination particular attention was directed to degenerative changes in the fiber substance of the valves, to proliferative reactions on the part of the stroma or endothelial cells, and to degenerative and proliferative lesions in the muscle and pericardium. Findings in the heart valves will be considered first.

The Normal Heart Valves

The normal heart valves present a characteristic structure. Eight control animals and, because in most instances they were normal, 17 animals subjected to uncomplicated infection, comprise the material for this analysis. Sections of the heart were obtained in all. Examination may be considered adequate in about 20 instances.

Control Animals: The normal valves as seen in the control animals possess a compact rich fiber structure. The fibrous stroma is closely set and consists of abundant, wavy, uninterrupted fibrils. The nuclei are of a mature sort, are regular in size and contour and are usually arranged axially. Little or no cytoplasm is visible around the nuclei, which appear to be naked in the fibrous stroma. Of the 8 control animals maintained on the basal diet supplemented with adequate amounts of orange juice none showed significant degenerative or proliferative reactions. Sections of each of these hearts were available for study and may be considered adequate in 5. In 1 animal a very mild proliferation of the endocardial layer was seen overlying the insertion of one of the large chordae tendineae.

Infection Only: The animals on the basal diet supplemented with orange juice and experimentally infected with *beta* streptococcus presented essentially normal valves, except as noted below. One animal showed an acute necrotizing mitral valvulitis, the second an accumulation of a few polymorphonuclear leukocytes near the base of the tricuspid valve with some associated capillary endothelial hyperplasia. Another animal declined with uremia from an obstructed urinary tract. In this animal there was a peculiar degeneration of the collagen fibers of a sort different from that to be described. In a few other instances there was observed a scattering of free mononuclear cells (one with a very mild interstitial proliferation) in the mitral valve and questionable foci of edema or degeneration. It could not be determined with certainty if these were true areas of edema, degeneration or artefacts. No associated proliferative reactions were seen, except the very mild reaction noted above. Of 20

animals in this group sections of the heart were secured in all and sections could be considered reasonably adequate in 15 instances. No changes were seen comparable in type or extent to those that occurred in the groups described below. Figure 1 illustrates an essentially normal aortic valve.

The Heart Valves in Scurvy

In practically all of the animals subjected to acute, severe subacute, or chronic scurvy definite degenerative changes in the structure of the valves are noted. The normally rich fibrous structure becomes impoverished. The fibers show thinning, fragmentation and disorganization of their regular axial arrangement. A loss of the normal wavy contours and at times a hyaline degenerative change of the fiber substance is observed. Eight of 9 hearts adequately examined in uncomplicated scurvy reveal changes of this sort.* Another animal in which but few sections are available also shows degenerative lesions. Nuclei of the stroma cells frequently present pyknotic shrunken contours. In addition to the collagen atrophy and degeneration described, in at least 2 instances a mild but definitely proliferative reaction of the endothelial or subendothelial cells is observed in uncomplicated scurvy.

Figure 2 illustrates the typical degenerative changes described, together with a mild proliferative reaction on the part of the endothelial and subendothelial cells. This is the most definite proliferative reaction that is seen in uncomplicated scurvy. Usually only the degenerative changes are present.

The Heart Valves in Combined Scurvy and Infection

The lesions produced in the heart valves in combined scurvy and infection are striking; they are characterized by degenerative changes with large cell proliferative reactions on the part of the stroma cells. Frequently masses or fragments of peculiar hyaline material are intermingled with the reacting cells. Fundamentally the lesions produced are similar, though differing individually in precise histology.

* This is in agreement with the fundamental observations of Hojer [2] who, although he did not describe the heart valves, observed atrophic connective tissue with poorly developed or imperfect collagen in various organs of animals subjected to scurvy. He observed these changes in early and relatively mild degrees of scurvy.

Scurvy and infection were combined in various ways as indicated in the experimental outlines and appended protocols. Of 31 animals subjected to one or another combination of scurvy and infection with *beta* streptococcus reasonably adequate examination of the heart was secured in 24 instances. Of this group all show recognizable degenerative changes in one or more heart valves.* In addition to the degenerative changes 16 hearts show moderate or marked proliferative reactions on the part of the stroma cells in one or more valves. Gross examination of the guinea pig's heart is difficult and unsatisfactory. However, the valves were examined in a few instances. Figure 3 shows a rim of elevated lesions along the line of closure of the mitral valve in an animal subjected to infection followed by scurvy. Figure 4 illustrates a leaflet of an aortic valve showing a diffuse swelling of the valve with a mucoid degenerative appearance of the stroma and a distinct nodular proliferative reaction at the line of closure. In the region of the greatest proliferative activity fragments of an eosinophilic hyaline material are visible. Figure 5 shows an essentially similar reaction in the mitral valve of another animal. Here again hyaline fragments are seen about the proliferating cells and a small, newly formed capillary is present in the midportion of the valve. In one instance, Figure 6, the proliferative reaction is unusually intense, overshadowing any degenerative changes. In another portion of this valve, however, shown in Figure 7, a definite hyaline degeneration is apparent in conjunction with a large cell proliferative reaction. The character of the proliferating cells is shown well, and it may be seen that some of them are multinucleated. Figure 8 illustrates a lesion in which a large amount of hyaline material lying within the substance of the mitral valve, together with the associated proliferation, forms a verrucous lesion at the line of closure. Figure 9, a higher magnification of the same valve, shows the dark, homogeneous eosinophilic hyaline material, the paler mucoid material and the proliferating cells, which make up the lesion. In Figure 10 is shown the mitral valve in an animal subjected to partial scurvy and infection. An area of hyaline degeneration with an accompanying proliferative reaction is seen in one leaflet. Here the endothelial surface layer has been eroded. There is a small focus of disintegrating red cells just beneath the surface. The

* In this study the mitral valve was most closely examined and in most though not all instances showed the maximal involvement.

distal portion of the valve shows atrophic degenerative changes in the collagen. The opposite leaflet presents hyaline degeneration with a mild diffuse hypertrophy and hyperplasia of the stroma cells. A striking lesion of the aortic valve is seen in another animal of the series, Figure 11. Here we find the normal fibrous structure is gone and replaced by cells with a large cytoplasm, some of which show two or more nuclei. At the contact surface is a thin fibrinous mesh. The two leaflets appear almost fused. Numerous fragments of an unidentified hyaline material resembling swollen collagen may be seen in the substance of the valve beneath the contact surface. The cytological characters of the proliferated cells are shown in Figure 12, taken at the contact area of the same valve. The proliferated cells in the various lesions present certain characteristic appearances: They show one or more large hyperchromatic or vesicular nuclei with sharp nuclear membranes and frequently prominent nucleoli or chromatin dots and ridges. The cytoplasm takes a dull eosinophilic or basophilic stain, is usually considerable in amount and outlined poorly, yielding cells of various shapes and irregular contours.

Similarity of the Experimental Lesions to those of Rheumatic Endocarditis

Attention is called to the similarity of the experimental lesions described to those of the early stages of rheumatic fever. Swift [3] has directed attention to the interstitial position of the fundamental lesion in rheumatic endocarditis. Clawson [4] has emphasized the essential proliferative nature of the inflammatory process in the valves in rheumatic endocarditis. In Poynton and Schlesinger's recent review of rheumatism [5] they state: "Evidence is rapidly accumulating in favor of Poynton and Paine's view that the valvular vegetations owe their formation to a subendothelial proliferation of cells and not to a primary destruction of the endothelium." Ribbert [6] speaks of characteristic changes in the subendocardial tissues beneath the thrombus or beneath the free surfaces, consisting in an enlargement of and gradual increase in number of the cells, whose cytoplasm enlarges and consequently causes a mild thickening of the tissues. This change, he finds, is accompanied by a transformation of the *zwischen-substanz* which becomes clearer, more translu-

cent and the fibrillar structure less distinct. The essential similarity of the experimental lesions to the early lesions of rheumatic fever is apparent. Perhaps the most exhaustive study of the pathology of rheumatic fever has recently been made by Klinge and his collaborators in a series of twelve papers on "Das Gewebsbild des fieberhaften Rheumatismus." The findings have been summarized in the last publication.[7] The fundamental lesion they describe as "a swelling of the ground substance of the connective tissue with a simultaneous chemical transformation into a substance which for the most part takes the stain of fibrin." This change, which they briefly designate as fibrinoid degeneration, is considered the primary lesion of rheumatic fever. In the heart valves, as in other connective tissues, there follows a proliferation of the connective tissue cells which, with a gradual disappearance of the fibrinoid masses, dominates the picture so that a part or the whole of the valve is transformed into a cell-rich granulation tissue. This comes close to the pathology observed in the experimental animals subjected to scurvy and infection. Klinge describes as classically rheumatic the verrucous lesion formed by an edematous, mucoid, hyaline, fibrinoid swelling of the valvular tissue (particularly the subendothelial layer), which pushes the endothelium before it forming a warty nodule. The above appears to describe perfectly the experimental lesion illustrated in Figures 8 and 9. This lesion is strikingly like a verrucous nodule shown in Figure 5 of Clawson's [4] study of endocarditis. Attention is called to the nuclear and cytoplasmic characteristics of the proliferating cells, as shown in Figures 7 and 12, which answer very closely the descriptions given by Gross, Loewe and Eliasoph [8] of the typical cells in the Aschoff reaction.

We have been unable to date to examine lesions extensively for the presence of bacteria. However, the Giemsa stain, on Zenker-fixed tissue, was applied in one of the most intense proliferative reactions and microörganisms could not be demonstrated.

Briefly, in summary, it may be said that animals subjected to combined scurvy and infection with *beta* streptococcus develop, in a considerable proportion, lesions in the heart valves resembling closely the lesions of rheumatic endocarditis.

Interstitial Reactions: The Aschoff reaction in its most characteristic form is perhaps pathognomonic of rheumatic fever. It is well to remember however, as has been noted by Gross and Ehrlich,[9] Swift,[10]

Clawson,[11] Klinge,[7] and others, that the Aschoff body is not a static structure but goes through an evolutionary cycle and that a considerable factor of time is probably necessary for the development of the lesion in its most characteristic form. In principle and character the lesions produced in the heart valves are fundamentally similar to those of the Aschoff reaction. Judged by the most severe standards it perhaps cannot be fairly said that the Aschoff body has been reproduced in the heart muscle. However, proliferative lesions have been observed in the myocardium and beneath the mural endocardium in animals subjected to a combination of scurvy and infection, which bear a strong resemblance to and are believed to be fundamentally similar to reactions seen in rheumatic fever. These reactions are clearly different from the accumulations of lymphocytes with or without large mononuclear cells and foci of more or less mature fibroblasts occasionally encountered in control animals, or in animals with infection maintained on an adequate diet. Characteristic proliferative lesions produced experimentally in combined scurvy and infection are illustrated in Figures 13, 14 and 15. Degenerative changes are usually discernible in the near-by muscle fibers. Figure 13 shows a well defined proliferative lesion at the angle of attachment of the mitral valve, a frequent site of lesions in rheumatic fever. Figure 14 shows a proliferative focus beneath the mural endocardium. In one area a focus of eosinophilic hyaline material resembling swollen fragmented collagen is seen, and in another area there is a scattering of red blood cells. Figure 15 shows a large cell proliferative response in the muscle of an animal infected and then maintained 38 days on a low vitamin C diet. The fibrinoid degeneration of Klinge has been encountered in the experimental animals in several situations; notably in the synovial and capsular tissues of joints, about the costochondral junctures, in a subcutaneous nodule in one animal and in the interstitial tissues of the heart in another. The latter lesion is shown in Figure 16. Finally, well defined proliferative reactions of large, and at times multinucleated, cells have been observed in the pericardium in several instances, occurring both in uncomplicated chronic scurvy and in scurvy with superimposed infection.

Experiments with B. Aertrycke: The experimental studies using organisms other than the *beta* streptococcus are not sufficient for final conclusions but a few observations with *B. aertrycke* as the

infecting agent suggest that the factor of infection in production of lesions is not specific. Two animals were maintained on the basal diet and subjected to infection by subcutaneous inoculation with *B. aertrycke* 18 days after onset. One animal surviving the infection only 6 days showed some atrophy and collagen degeneration and a small focus of proliferation in the mitral valve. The second animal survived infection 20 days and showed collagen degeneration, a distinct proliferative reaction at the base of the mitral valve, and an Aschoff-like reaction in the muscle at the region of attachment of the mitral valve. An animal, given an adequately supplemented diet and subject to the same type of infection for a similar period, showed essentially normal valves. Two animals were subjected to severe but not total scurvy and infection by intravenous inoculation with *B. aertrycke*. One developed an acute necrotizing and hemorrhagic mitral valvulitis, the other showed definite collagen degeneration and a diffuse interstitial proliferative reaction in the mitral valve. Unfortunately no sections were secured in the 2 control animals of this series. The data for this organism remain incomplete and inadequate but suggest that the infection need not necessarily be specific.

Discussion

Experimental Attempts to Reproduce Rheumatic Fever: Experimental attempts to reproduce the disease rheumatic fever have proved disappointing. No attempt will be made to review the rather extensive literature since the subject has been summarized recently by Gross, Loewe and Eliasoph,[8] who themselves conducted rather elaborate experiments in an attempt to reproduce rheumatic fever. They conclude: "Judged by the criteria used, we have failed to reproduce the disease. This conclusion we believe holds true for all the work thus far reported in the literature." Studies for the most part have been made with a variety of streptococci isolated in various ways from cases of acute rheumatic fever. While it is true that endocarditis and arthritis have been produced by injecting large doses of streptococci intravenously, such procedures obviously differ rather radically from clinical conditions of rheumatic fever. Although organisms may be found at times in the blood stream in acute rheumatic fever there is no evidence of overwhelming bacteremia. The lesions produced experimentally by such massive intravenous

inoculations with streptococci have corresponded more closely to vegetative endocarditis and suppurative arthritis than to those of rheumatic fever. It is believed that the lesions produced in the heart valves, muscle and pericardium by the experimental method employed in this study correspond more closely to those of rheumatic fever than any previously reported.

The Similarity of the Fundamental Pathology of Scurvy and Rheumatic Fever: Numerous investigations, notably those of Aschoff and Koch,[12] Hojer,[2] and Wolbach and Howe,[13] have revealed the basic defect of the scorbutic animal to be an inability to form normal intercellular substances, such as bone, dentine, collagen, and so on. Degenerative changes overtake the preformed collagen and the organism is unable to replace it with a normal substance. The reparative attempt is abortive and an imperfect cement substance is formed. It is of great interest to note that the concept is developing that the fundamental lesion of rheumatic fever is an abnormality of, or degenerative change involving, the intercellular substance of the connective tissue. This has been stressed by Klinge and described as a fibrinoid swelling of the ground substance. It has been previously noted that such lesions corresponding closely to Klinge's descriptions and illustrations were observed in various sites in the experimental animals. Shaw [14] and others have pointed out that masses or fragments of swollen collagen are practically always demonstrable at the center of the rheumatic proliferative reactions. It is quite clear that the dominant lesions of rheumatic fever, *i. e.*, endocarditis, arthritis and subcutaneous nodules, occur at points of stress. It would be at such sites that any weakness or imperfection in the intercellular substance of the connective tissue would first become manifest. It would appear from the experimental studies that the infection alone or the scorbutic state alone are in themselves not adequate for development of the characteristic lesions of rheumatic fever, but that when the insult of infection is added to the fundamental disability of the scorbutic state the lesions occur.

The Factor of Infection in Rheumatic Fever: The vast amount of clinical and bacteriological data that have accumulated would appear certainly to indicate a factor of infection in rheumatic fever and almost certain to implicate one or another form of streptococci. The bacteriological observations on rheumatic fever are extensive. The essential contributions to the bacteriology of this disease are avail-

able in recent reviews by Coburn,[15] Swift,[10] and Poynton and Schlesinger.[5] All studies point strongly to streptococci, but there is no uniformity in the type or strains described. To explain rheumatic fever simply on the basis of streptococcic infection is inadequate in several respects, notably the unique pathology of the disease, the failure to reproduce the disease experimentally with simple streptococcic infection and finally the lack of uniformity in the type and strain of organisms found associated with it. With a concept of a condition of vitamin C undernutrition as the essential background for rheumatic fever the need for a strain specific infection would appear unnecessary.

Recent investigations of Coburn,[15] Swift,[10] and Gibson and Thomson [16] point rather strongly to the practical importance of *Streptococcus hemolyticus*. Coburn [15] recently studied and reviewed the factor of infection in the rheumatic state. He has observed the prevalence of *Streptococcus hemolyticus* infection in New York in the spring months and makes the following significant statement. "When there was actual local disease resulting from the infection with *Streptococcus hemolyticus*, the clinical picture was manifest as scarlet fever, pharyngitis, tonsillitis, sinusitis, otitis, mastoiditis, cervical adenitis or bronchopneumonia, depending upon the tissue involved and the nature of the clinical response. In most individuals the local disease was the only manifestation of invasion of the tissues with *Streptococcus hemolyticus*. In a small minority, however, local infection was followed by the rheumatic state." This, together with observations by many others, suggests an undefined state occurring in certain individuals which predisposes them to rheumatic fever. Gibson and Thomson find an association between infection with *Streptococcus hemolyticus* and rheumatic fever, but state: "There is some other factor as yet elusive which is required to complete the picture." The observations here reported, together with other preliminary experimental observations on the joints and a study of the epidemiological peculiarities of rheumatic fever, [17] notably the social and seasonal incidence and geographic distribution, strongly suggest that a nutritional state of latent or submanifest scurvy may be the factor predisposing certain individuals to develop rheumatic fever under the added stress of infection. That vitamin C malnutrition exists as a problem of definite importance has been indicated by the work of Göthlin [18] in Sweden, and Dalldorf [19] in this country.

Representative Protocols

Series I

Control: No. 66. Put on basal diet 8/1/32. Weight 300 gm. Given orange juice supplement 4 cc. daily by pipette. Excellent condition when chloroformed on 8/29/32. Weight 375 gm.

Pathological notes: Tissues normal.

Infection Only: No. 61. Put on basal diet 8/1/32. Weight 375 gm. Given daily adequate orange juice supplement. 8/16/32, infected with *beta* streptococcus 0.1 cc. intracutaneously in thigh (inner aspect). 8/19/32, small pustule and regional enlarged lymph node. 8/30/32, skin healed, 2 small, regional lymph nodes. 9/6/32, chloroformed.

Pathological notes: Encapsulated suppurated inguinal lymph nodes. Other tissues essentially normal.

Total Scurvy: No. 49. Put on basal diet 8/1/32. Weight 300 gm. Maintained without orange juice supplement until death 9/2/32. Weight 190 gm.

Pathological notes: No evidence of infection. Collagen degeneration and slight proliferation in mitral valve (Fig. 2). Hemorrhage into bladder. Erythrophagocytosis in lymph nodes.

Total Scurvy Plus Infection: (*beta* streptococcus) No. 53. Placed on basal diet only 8/1/32, weight 340 gm. 8/16/32, weight 390 gm. Inoculated intracutaneously skin of thigh with 0.1 cc. 24 hour culture *beta* streptococcus. 8/19/32, pustule with small regional lymph node. 8/26/32, large local mass of lymph nodes with reddened, fixed overlying skin. 9/5/32, animal given 2 cc. orange juice. 9/6/32, sacrificed. Weight 210 gm.

Pathological notes: Necrotic area in left groin surrounded by enlarged infected lymph nodes fixed to surrounding tissues. Mitral valve shows typical verrucous lesion (Fig. 8). Spleen enlarged, hyperplastic pulp, microscopic abscesses, early fibrosis of malphigian bodies. Lungs, marked passive congestion and edema. Hemorrhage into adrenals and bladder. Costochondral junctures show typical scorbutic changes, one microscopic focus of osteomyelitis. Fibrinoid degeneration in heart and about costochondral junctures.

Subacute Scurvy Plus Infection: (*B. Aertrycke* intravenously) No. 67. Placed on basal diet 8/18/32. So maintained without orange juice for 21 days. Then partial scurvy for the next 21 days to 9/28/32 (total 14 cc. orange juice in divided doses). 9/1/32, inoculated into the skin with *alpha* streptococcus (human source). Did not develop any infection. 9/14/32, 0.2 cc. of broth culture of *B. aertrycke* given intravenously. Died 14 days later on 9/28/32.

Pathological notes: Abscess in liver, fibrinous pleurisy with collapse of left lung. Hemorrhage into adrenals and swollen costochondral junctures. Microscopic examination shows a degenerative and proliferative lesion in the mitral valve and a slight pericardial proliferative reaction; purulent empyema with subpleural abscesses; focal necrosis in the liver; fibrosis of malphigian bodies in the spleen.

Series II

This series was essentially similar to Series I. The infecting agent was a *beta* streptococcus. The group of animals was younger and the pathological changes less definite, due to the shorter survival period of younger animals.

SERIES III

Infection Only: (*beta* streptococcus) No. 105. Placed on basal diet 12/1/32. Weight 330 gm. Received daily orange juice supplement throughout experiment. 12/15/32, inoculated intracutaneously (left thigh) with 0.1 cc. broth culture *beta* streptococcus. 12/21/32, showed small nodule at site of inoculation. No involvement of regional node. Reinoculated right thigh with 0.1 cc. broth culture *beta* streptococcus from another infected animal. Developed skin reaction and regional lymph node infection, which reached maximum size of 1.5 cm. by 1 cm. on 12/27/32. Also a smaller node in the right groin. Subsequent course: regression of lesions. Chloroformed 1/31/33.

Pathological notes: Encapsulated lymph node 0.5 cm. in diameter in left groin. Other gross findings normal. Questionable edema in leaflet of mitral valve. Focal accumulation of lymphocytes in heart muscle.

Subacute Scurvy Plus Infection: (*beta* streptococcus) No. 111. Placed on basal diet without orange juice 12/1/32. Weight 315 gm. 12/15/32, inoculated intracutaneously 0.05 cc. 28 hour broth culture *beta* streptococcus, left thigh (inner aspect). 12/21/32, small lymph node enlargement. Reinfected with 0.1 cc. broth culture of *beta* streptococcus of different source by intracutaneous inoculation in right thigh. Developed enlarged regional lymph node, which subsided after maximum enlargement on 12/27/32. Both knees swollen 12/24/32. Beginning 12/19/32 over period of 11 days was given total of 18 cc. orange juice in divided doses. 1 cc. given 1/4/33 and none further until chloroformed 1/20/33. Weight 254 gm.

Pathological notes: Gross findings — enlarged spleen, moderate hemorrhage about knees and into chest wall on left. Swollen costochondral junctures. Gross examination of mitral valve showed verrucous nodule. Microscopic examination showed degenerative and proliferative lesion of mitral valve (shown in Fig. 10). Spleen shows hyperplastic pulp, erythropoiesis and fibrosis of malphigian bodies.

Chronic Scurvy Plus Infection: (*beta* streptococcus) No. 118. Weight 365 gm. Placed on basal diet 12/1/32. Duration of experiment 2 months. Received 2 cc. orange juice on alternate days for first month and 1 cc. on alternate days for last month. 12/15/32, inoculated 0.05 cc. culture *beta* streptococcus, intracutaneously left thigh. Developed small skin lesion and small node in groin. 12/21/32, reinoculated right thigh 0.1 cc. *beta* streptococcus. 12/27/32, shows large infected mass in the left groin fixed to surrounding tissue and skin and immobilizing the knee. Smaller lesion on right. Gradual regression of lesions until sacrificed 1/31/33.

Pathological notes: No evidence of active infection. Knees fixed. Nodule noted grossly on aortic valve. Costochondral junctures swollen. Microscopic examination shows striking proliferative lesion on aortic valve (Fig. 11). Fibrinoid degeneration about costochondral junctures. Degenerative changes in intercostal muscle. Fibrosis of malphigian bodies in spleen. Fibrinoid degeneration and proliferation in synovia and periarticular tissue of knee. Little hemorrhage into joint spaces.

SERIES IV

Dietary Control: No. 130. Weight 368 gm. Maintained on basal diet supplement with orange juice (4 to 6 cc. daily) for 71 days when chloroformed. Weight 625 gm. Excellent condition. Joints negative.

Pathological notes: No gross changes. Heart valves normal limits. Slight endothelial proliferation over insertion of one of the large chordae tendineae. Focal accumulation of lymphocytes and nuclear fragments in tissue between auricle and ventricle. Knees histologically normal.

Chronic Scurvy Only: No. 149. Weight 390 gm. Placed on basal diet beginning 1/19/33. Maintained for 71 days with 24 cc. orange juice in divided doses. 2/23/33 showed partial fixation of both knees. Chloroformed 3/31/33. Weight 300 gm.

Pathological notes: Slight hemorrhage into chest wall. Hemorrhagic stippling of bladder. Swollen costochondral junctures. Little hemorrhage into muscle above and below knees. Mitral valve shows moderate degeneration. A small Aschoff-like proliferative reaction is seen in the epicardium. Lymph nodes show erythrophagocytosis and pigmentation. Fibrinoid degeneration about costochondral juncture. Fatty degeneration about central veins of the liver.

Infection Only: (*beta* streptococcus) No. 132. Weight 411 gm. Placed on basal diet beginning 1/19/33. Maintained for total of 67 days with daily orange juice supplement (4 to 6 cc.). Inoculated 2/10/33 with 0.1 cc. broth culture *beta* streptococcus 22 days after onset. 2/19/33, shows enlarged inguinal lymph node 1.2 by 0.6 cm. Gradual regression of infection until sacrificed 3/27/33. Weight 550 gm.

Pathological notes: Complete regression of local infection. Organs and tissues including joints grossly normal. Microscopic examination: heart valves normal except for questionable area of degeneration in mitral valve. Excess iron pigment in spleen and old degenerative lesion at costochondral juncture (evidence of old scurvy anteceding experiment?). Knee and elbow joints quite normal.

Chronic Scurvy Plus Infection: (*beta* streptococcus) No. 152. Weight 408 gm. Dietary regimen as in No. 149. Infection as in No. 132. Basal diet for 67 days with total of 24 cc. orange juice in divided doses. Inoculated 0.1 cc. broth culture *beta* streptococcus into skin of left thigh on 2/10/33. 2/19/33, infection maximal with large mass in left groin 2 by 1.3 cm. On this day noted swelling and fixation of right knee and fixation of left knee. Infection persistent, slow regression. Animal chloroformed 3/27/33. Weight 272 gm.

Pathological notes: Small lymph nodes in both groins. Patchy atelectasis of lungs. Costochondral junctures swollen. Single abscess in spleen. Bladder hemorrhagic. Brownish discoloration (old hemorrhage) about right knee. No evidence of recent hemorrhage. Questionable shortening of mitral valve. Subcutaneous nodule over bony prominence of knee. Microscopic examination shows definite interstitial proliferation in mitral valve, shown in Fig. 5. The subcutaneous nodule shows bands of fibrinoid degeneration and endothelial proliferation resembling the lesion of rheumatic fever. Joints show fibrinoid degeneration in synovia and periarticular tissues. Similar degeneration in connective tissues about costochondral junctures. Much hemosiderin in the spleen. Hemosiderin and erythrophagocytosis in cervical lymph nodes.

Infection Followed by Subacute Scurvy: No. 142. Placed on basal diet 1/19/33 and so maintained with adequate orange juice supplement (4 to 5 cc. daily) to 2/10/33, when inoculated with 0.1 cc. culture *beta* streptococcus. Following this, maintained for 40 days with total of 11 cc. orange juice in divided doses. Maximal local infection 2/25/33. Mass in left groin 1.8 by 1 cm.

3/3/33, right knee slightly fixed and swollen. 3/14/33 both knees fixed and swollen. 3/18/33, elbows swollen and tender. 3/22/33, sacrificed. Weight 272 gm.

Pathological notes: Some hemorrhage into chest and abdominal walls and into bladder. Swollen costochondral junctures. Heart: gross lesion mitral valve, Fig. 3; microscopic lesions Figs. 6 and 7. Spleen: pigment in pulp and fibrosis of malphigian bodies. Hyaline material in joint spaces with synovial proliferation. Proliferation of capsular tissues.

Summary

The effect of scurvy, and scurvy combined with infection (*beta* streptococcus), upon the heart valves and muscle in the guinea pig has been studied. Infection in animals maintained on an adequate diet usually produces no significant lesions in the heart valves. When they occur they are of an exudative, rather than proliferative type. In uncomplicated scurvy definite atrophic and degenerative changes occur in the collagenous stroma of the heart valves. In scurvy with added infection striking lesions of a combined degenerative and proliferative character develop in the heart valves with considerable frequency. Attention is directed to the similarity of the experimental endocarditis so produced to that of acute rheumatic fever.

Degenerative and proliferative lesions occur in the heart muscle and pericardium of the experimental animals subjected to combined scurvy and infection which are considered similar in type to the Aschoff reaction.

Brief evidence is given that organisms other than the *beta* streptococcus may, in the presence of scurvy, produce such lesions.

The similarity of the fundamental pathology of scurvy and rheumatic fever is considered. Attention is called to the fact that a basic lesion in both conditions is a degenerative change occurring in collagen. Lesions resembling the "fibrinoid degeneration" of Klinge have been seen in various sites in animals subjected to combined scurvy and infection. Klinge considers this type of degeneration the characteristic and initial lesion of rheumatic fever.

The role of infection in rheumatic fever is briefly discussed. It is pointed out that a factor of infection would appear beyond dispute and that the streptococcus in one form or another is strongly implicated. Much evidence clearly indicates that there is some other

factor than simple infection contributing to the development of rheumatic fever.

Experimentally, infection alone or scurvy alone will not produce significant lesions, but when scurvy and infection are combined striking lesions are produced, particularly in the heart valves.

On the basis of the experimental data reported, and other experimental, epidemiological and clinical data previously outlined, the theory is advanced that a condition of vitamin C undernutrition may be a necessary background for the development of rheumatic fever; when the insult of infection is combined with the scorbutic state, the pathological picture of rheumatic fever develops.

NOTE: The authors wish to express appreciation to Dr. Charles L. Connor for stimulation and advice given during the course of these experiments.

We also wish to acknowledge the valuable technical assistance of Dr. L. L. Ginzton, Mrs. K. L. Purviance, Miss Pearl Y. Hall, Mr. W. A. Hewitt and Mr. E. Nauman. Miss Bernice Eddie generously assisted in the bacteriological work.

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DESCRIPTION OF PLATES

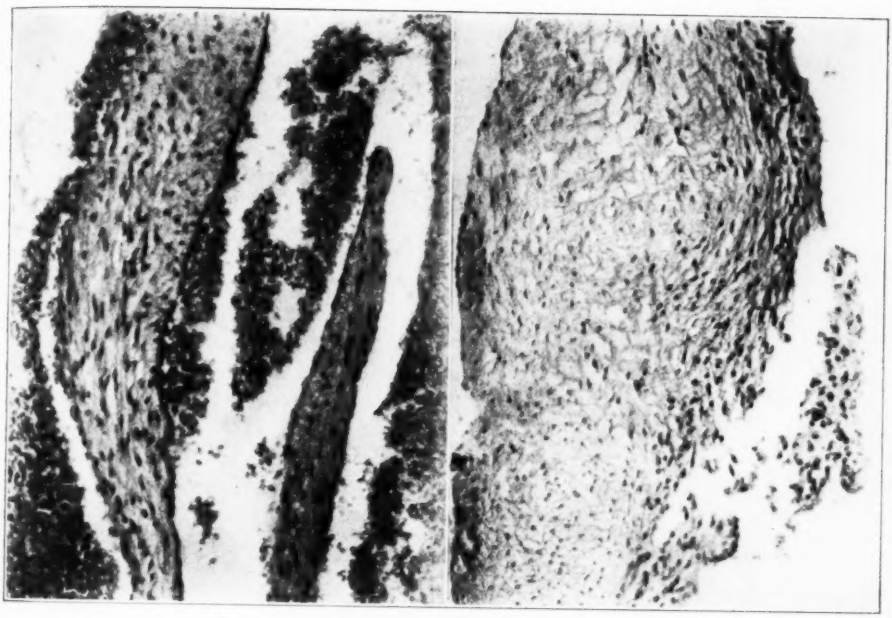
PLATE 43

FIG. 1. Aortic valve. Control infection. Animal No. 132. Showing compact structure of essentially normal aortic valve. × 95.

FIG. 2. Mitral valve. Scurvy only. Animal No. 49. Showing degenerative changes in valvular stroma and a mild proliferative reaction. The degenerative changes are characteristic; proliferative reactions are not usual in uncomplicated scurvy. × 90.

FIG. 3. Mitral valve. Infection plus scurvy. Animal No. 142. Showing rim of elevated nodular lesions along line of closure of the mitral valve. Drawn under dissecting microscope. See Figs. 6 and 7 for microscopic appearances of this valve.

FIG. 4. Aortic valve. Chronic scurvy plus infection. Animal No. 159. Showing swelling and degeneration of valve with distinct proliferative nodule at line of closure. × 100.



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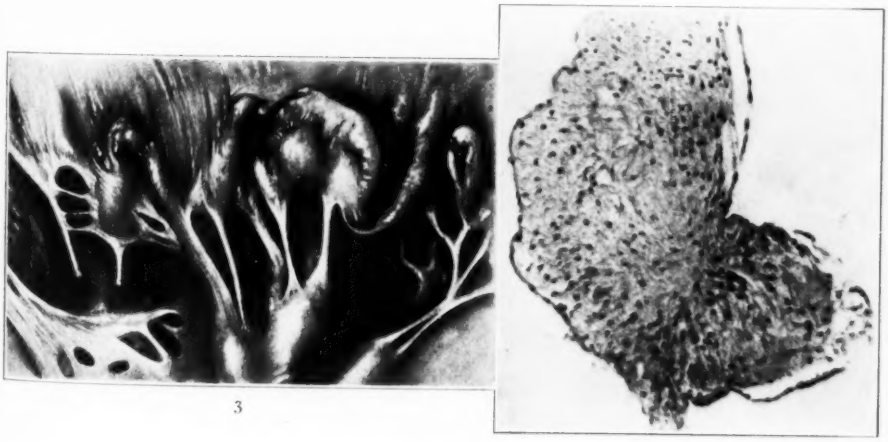


3 4

Rinehart and Mettier Heart Valves and Muscle in Experimental Scurvy

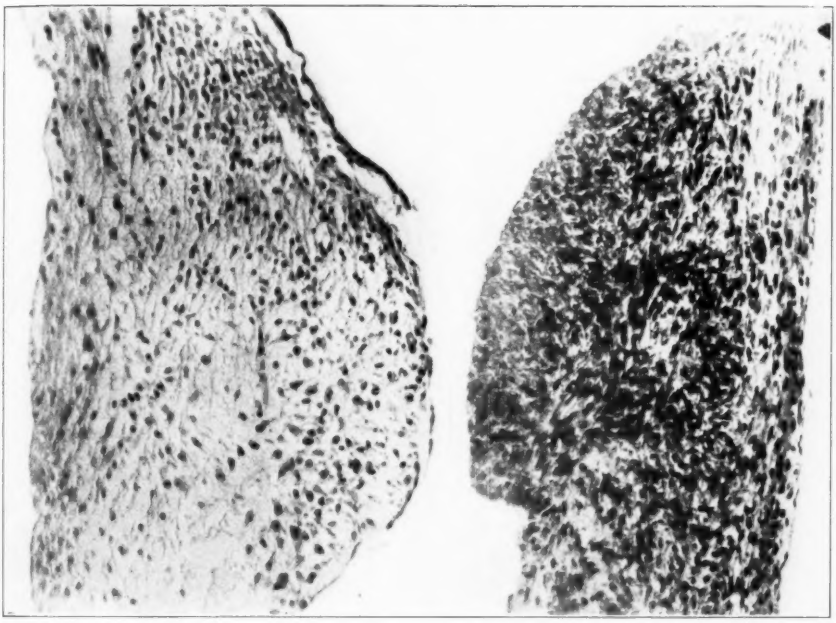
PLATE 44

FIG. 5. Mitral valve. Chronic scurvy plus infection. Animal No. 152. To show swelling and degeneration in the stroma and a diffuse subendothelial proliferative reaction in the mitral valve. A delicate, newly formed capillary may be seen in the mid portion of the valve. × 120.

FIG. 6. Mitral valve. Infection plus scurvy. Animal No. 142. An unusually intense proliferative reaction involving the full thickness of the valve. See Fig. 3 for gross appearance. × 95.

FIG. 7. Mitral valve. Infection plus scurvy. Animal No. 142. Another area of the valve illustrated in Fig. 6. This section shows a hyaline degenerative change in the valve stroma with an accompanying proliferative reaction. Detail of the proliferated cells may be seen. Some are multinucleated. × 175.

FIG. 8. Mitral valve. Scurvy plus infection. Animal No. 53. A combined degenerative and proliferative lesion in the mitral valve occurring in region of line of closure. See next figure for detail. × 45.



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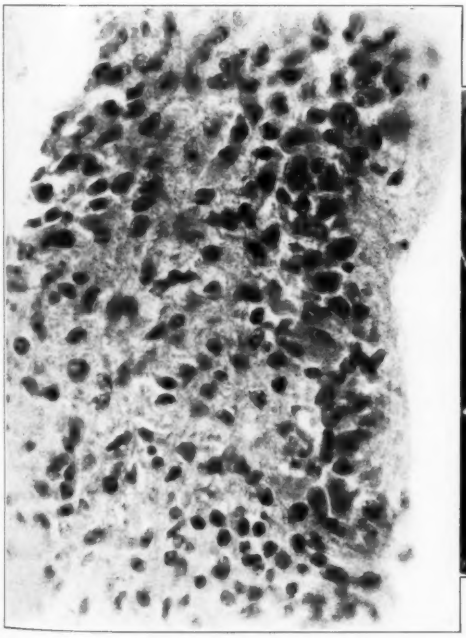

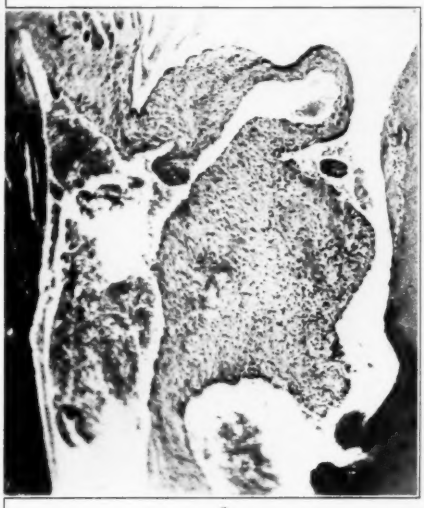

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Rinehart and Mettier Heart Valves and Muscle in Experimental Scurvy

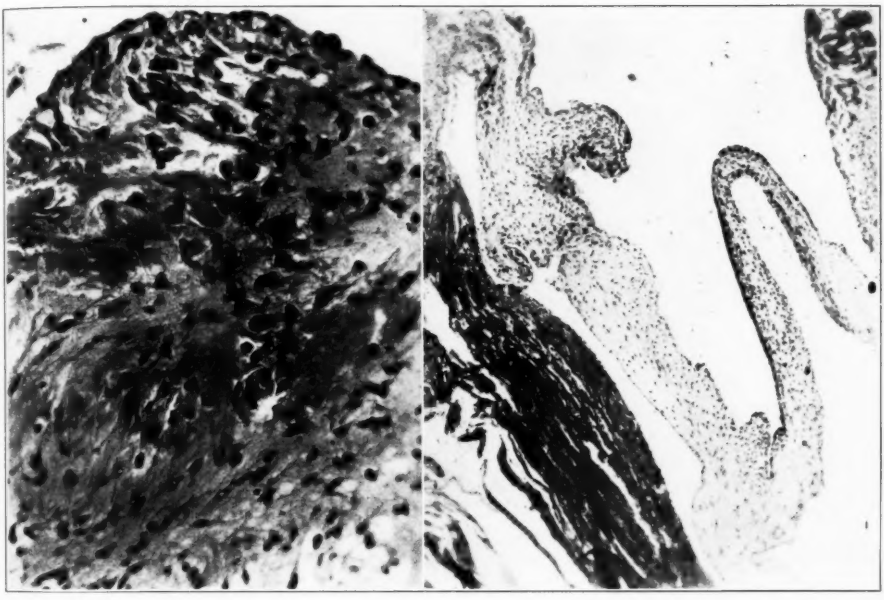
PLATE 45

FIG. 9. Higher magnification of the lesion illustrated in Fig. 8. A fibrinoid and mucoid degeneration with an early proliferative response composes this verrucous nodule. This lesion is considered characteristically rheumatic. × 350.

FIG. 10. Mitral valve. Subacute scurvy plus infection. Animal No. 111. An area of hyaline degeneration with an accompanying proliferative reaction is seen in one leaflet. Here the endothelial surface layer has been eroded. There is a small focus of disintegrating red blood cells just beneath the surface. The distal portion of this leaflet shows atrophic degenerative changes in the collagen. The opposite leaflet presents hyaline degeneration with a mild diffuse hypertrophy and hyperplasia of the stroma cells. × 45.

FIG. 11. Aortic valve. Chronic scurvy plus infection. Animal No. 118. A striking proliferative reaction in two leaflets of the aortic valve; diffuse edema of valve with proliferation of hyperplastic cells. The two leaflets appear almost fused. Fragments of an unidentified hyaline material resembling swollen collagen are present in the substance of the valve beneath the contact surfaces. A thin layer of fibrin lies between the two leaflets. × 95.

FIG. 12. Higher magnification of same lesion shown in Fig. 11. Showing detailed characteristics of proliferating cells. Note the large vesicular nuclei with prominent chromatin dots and nuclear membranes. The cytoplasm is abundant, dully eosinophilic (hematoxylin and eosin stain), faintly granular and irregularly outlined. Some of the cells show more than one nucleus in a single mass of cytoplasm. × 650.



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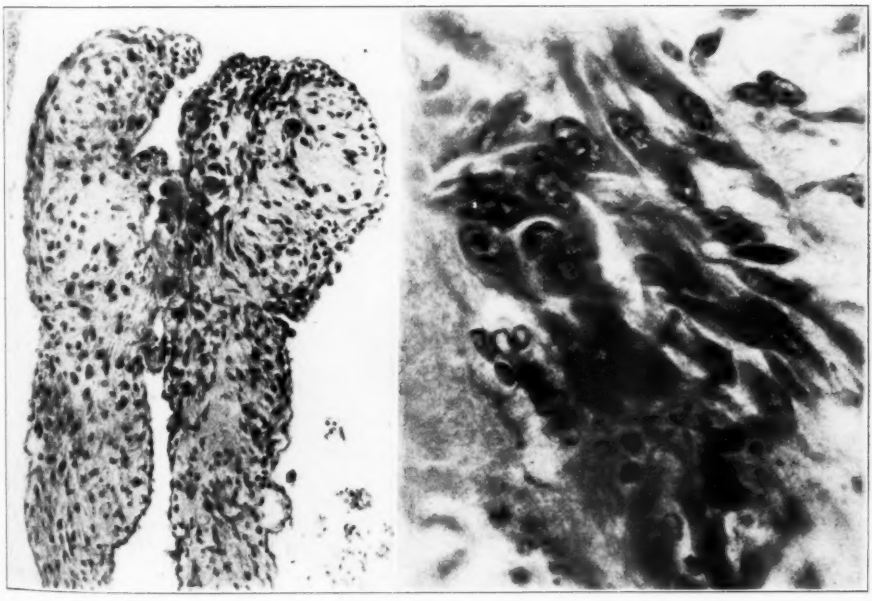

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Rinehart and Mettier Heart Valves and Muscle in Experimental Scurvy

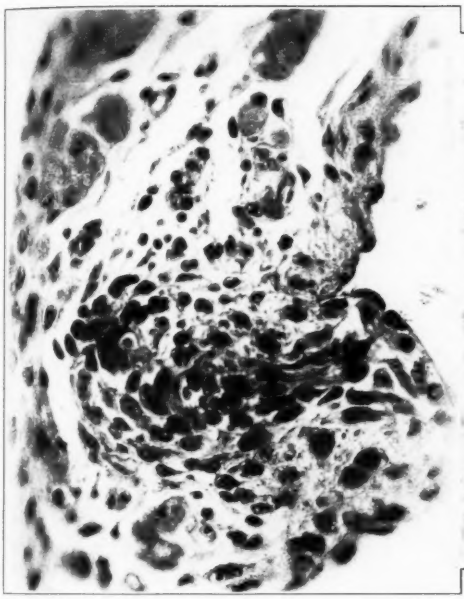
PLATE 46

FIG. 13. Interstitial proliferative reaction at the angle of attachment of the mitral valve. Infection plus scurvy. Animal No. 143. Showing a concentric proliferative nodule composed of hyperplastic cells resembling the Aschoff reaction of rheumatic fever. × 420.

FIG. 14. Subendocardial reaction. Chronic scurvy plus infection. Animal No. 115. This proliferative reaction beneath the mural endocardium shows in one area a focus of eosinophilic hyaline material resembling swollen collagen, and in another area there is a scattering of red blood cells. The hyperplastic cells resemble those of the Aschoff reaction. × 420.

FIG. 15. Interstitial proliferative reaction. Infection plus scurvy. Animal No. 145. Showing a portion of an interstitial proliferative reaction. The character of the cells forming the focus is well shown. Some contain more than one nucleus. Although not well shown in the photograph the surrounding muscle fibers show distinct degenerative changes. × 560.

FIG. 16. Fibrinoid degeneration of collagen in heart. Scurvy plus infection. Animal No. 53. A focus of degenerated collagen, which histologically reacts as fibrin, is shown adjacent to the heart muscle. The change involves the wall of a small vessel in addition to an elongated focus of collagen. A change of this type is considered by Klinge to be the fundamental and earliest lesion of rheumatic fever. × 175.



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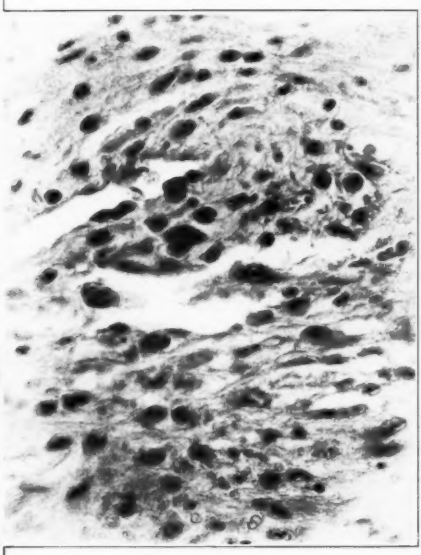

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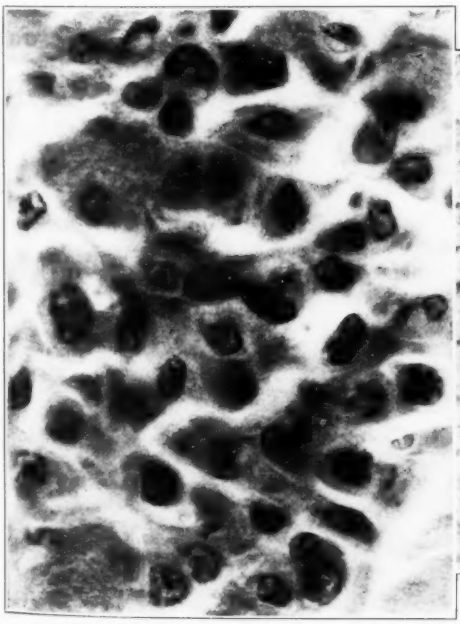

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16

UNIV. OF MICH.

REPORT OF A CASE OF NON-LIPOID HISTIOCYTOSIS (RETICULOENDOTHELIOSIS) WITH AUTOPSY *

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INTRODUCTION

During the past decade, following Aschoff's and Kiyono's promulgation of their conception of a reticuloendothelial system, a number of cases have been reported that appear to represent a stimulation of this system, without constituting neoplasia and without showing the lipoid changes of Gaucher's disease or of lipoid histiocytosis. Most of these have appeared in the German literature, coming from German or Russian sources. The titles of the articles vary from "Reticuloendotheliosis" to "Aleukemic Reticulosis," with the use of other terms to express the pathological pictures observed. Theories as to the nature of the disease fall into two categories: the conception that it represents a response to bacterial or toxic stimuli and is, therefore, of an infectious nature, and the idea that it constitutes a third type of leukemia in which the monocyte (or histiocyte) is the type cell. Cases have been reported in which there did appear to be a monocytic leukemia but, on the other hand, many articles have been published in which infection seems most important, and there is no mention of increase of mononuclears in the circulation. We have chosen the term "non-lipoid histiocytosis" as expressing a subdivision in the reticuloendothelioses in general and in contradistinction to lipoid histiocytosis and its nearly related condition, Gaucher's disease, in which lipoids are a prominent feature.

The clinical picture of the disease is very indefinite and poorly characterized. The age and sex of the patients seem to be unimportant, although the picture is somewhat different in childhood from its type in adult life. There is usually a history of recent infection, ranging from grippe or a severe cold, to otitis media, faucial infections

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such as Vincent's angina, or infections of the intestinal or genito-urinary tracts. The patients show a secondary anemia of varying grade and, in most cases, there is a slight leukocytosis of the polymorphonuclear type, although leukopenias may be noted, as in our case. Fever is usually not a prominent feature, although the temperature is often subfebrile. A history of frequent and sometimes familial epistaxis may be elicited. In children there is almost always a marked and generalized purpuric eruption on the skin with scattered, very small petechiae over the greater part of the body. For this reason thrombocytopenic purpura is often diagnosed, to be subsequently ruled out by further examination.

Physical signs are scanty, except for a variable lymphadenopathy and an almost invariably enlarged spleen. The liver is also usually found to be enlarged in the case of children, while in adults this is not always the case. On roentgenological examination bone changes are frequently seen; it is possible that they are more common than is supposed and could have been demonstrated had they been looked for with the X-ray. Three cases besides ours showed cysts or areas of rarefaction in the bones (Guizetti,[1] Goldzieher and Hornick,[2] and Schultz, Wermbter and Puhl[3]); Pentmann's[4] case showed numerous cavernous hemangiomas, one of which involved a vertebral body.

At autopsy the following pathological changes are usually noted. In children there is a generalized petechial skin eruption; petechial hemorrhages in the epicardium (as distinguished from the pericardium in general) are almost the rule in cases of all ages, and there may also be petechiae in the pleura. The thymus is the site of small abscess-like areas of softening resembling Dubois abscesses and referable to involution and necrosis of the Hassal's corpuscles, as determined by the microscope. The lungs and heart show nothing characteristic. There is usually generalized enlargement of the lymph nodes, the cervical, retroperitoneal and other chains often being involved as groups; sometimes the nodes at the portal region are very large. The liver is practically always enlarged in children and shows extensive fatty infiltration; in adults it may be enlarged, but is not regularly fatty. In both cases early cirrhotic changes are the rule. The spleen is always large, firm and tense, with rounded edges. On section the markings are indistinct and there are sometimes yellowish nodules in the parenchyma. The bone marrow often shows overgrowth into the bony spongiosa, with rarefaction of the

latter that sometimes goes on to cystic degeneration. The changes noted elsewhere in the body are variable and not characteristic.

Microscopically these changes are found to be due to an overgrowth of large mononuclear cells that multiply in the sinuses of the lymphoid apparatus and spleen to an almost neoplastic degree, at the expense of the lymph follicles, and often show marked phagocytic tendencies toward erythrocytes and leukocytes. Sometimes they merely contain hemosiderin or chromatin fragments. These large cells are characterized by a dense, sometimes basophilic cytoplasm, a polyhedral contour, a tendency to lie discretely separated and to form giant cells or syncytia that differ somewhat from those of the Langhans type or the Dorothy Reed (Sternberg) variety. Their nuclei are vesicular and correspond with those of the retothelium (we shall use Roulet's [5] abbreviated terminology for reticuloendothelium) in general. They may have one, or several of these. Often there are cells quite indistinguishable from megakaryocytes in both spleen and lymph nodes. None of the type cells is possessed of the clear, longitudinally meshed or striated cytoplasm of the Gaucher cell, or of the foamy, vacuolated body of the Pick-Niemann type. In some cases reported eosinophils and plasma cells have been noted in association with these retothelial cells in moderate or large numbers. The liver shows a similar, but usually much less marked, retothelial proliferation and infiltration in the sinusoids and the portal areas, together with a granulomatous overgrowth of lymphoid tissue in the latter. The Kupffer cells may or may not be involved in the process. The picture here is far less striking than it is in the spleen and lymph nodes. Overgrowth of the connective tissue of the liver is the rule, sometimes amounting to early cirrhosis (there may be central necrosis in adults); in children extreme and generalized fatty infiltration of the parenchyma is the rule. The bone marrow is also the site of similar retothelial overgrowth at the expense of hematopoiesis and there may be considerable increase in the fibrous tissue and vascular content of the marrow with an apparent congestion and hemorrhage. Sometimes there is a gelatinoid degeneration of areas of marrow that invade and destroy the spongiosa and may form cysts.

The origin of the type cells in this disease is generally attributed to the reticuloendothelial apparatus; to enter into a discussion of this would merely reopen a much discussed subject. The participation of the Kupffer cells in the process is usually assumed, but scarcely

proved as yet. We shall discuss the question of etiology later; thus far we have simply attempted to establish criteria for our diagnosis by reference to facts gleaned from the study of some twenty cases reported in the literature. It should be noted, in passing, that only one of these comes from an American source; either the condition is rare in this country, or our pathologists have overlooked it and failed to recognize it.

Report of Case

Clinical History: The patient was a 2¾ year old white female who had been ill for about 1 year, during which she had had red, hemorrhagic spots on the skin. Her father had suffered from hay fever and was subject to frequent nosebleeds, which was also true of her maternal grandmother; her father's brother had also had frequent epistaxis. The child was a normal, full-term baby. She had had three attacks of catarrhal otitis media and had had frequent colds, together with colitis on one occasion. The spleen had been noted as enlarged 3 months prior to admission and, at that time, she had had severe nasal hemorrhage. Shortly after this she was admitted to the Johns Hopkins Hospital, where a diagnosis of purpura hemorrhagica was made. The blood count showed: red blood cells 3,830,000, hemoglobin 75 per cent, white blood cells 4100, polymorphonuclears 25 per cent, lymphocytes 69 per cent. The smear showed few platelets, the bleeding time was 6 minutes, clotting time ¼ minute. Treatment consisted of a transfusion and the family was advised to have a splenectomy performed.

Upon admission to the Department of Pediatrics of the New York Hospital the child's body was found to be covered with tiny punctate petechiae. The liver was palpable 7 cm. below the right costal margin. The spleen was also palpable. The tonsils were large and the inguinal and posterior cervical lymph nodes moderately so. There had been no jaundice. Throat cultures and the Schick and Mantoux tests were negative. The temperature remained below 37.8° C. The first blood count showed: red blood cells 3,700,000, white blood cells 3900, adult polymorphonuclears 30 per cent, immature polymorphonuclears 7 per cent, lymphocytes 39 per cent, mononuclears 4 per cent, and eosinophiles 2 per cent. Two platelet counts showed 80,000 and 130,000 respectively. The clotting and bleeding time were 3 minutes with normal capillary clot. The day after admission it was noted on her chart: "Not a typical case of thrombocytopenic purpura, in view of the marked enlargement of the liver and spleen and the normal bleeding time and clot retraction." Roentgenological examination showed no changes suggestive of Gaucher's disease, but there was some stippling of the lower ends of both humeri.

The child was readmitted about 2 weeks later and a blood examination showed that the platelet count had fallen, while the liver had still further increased in size. Physical examination revealed nothing of note except for the enlarged liver and spleen. The lymph nodes were not much enlarged. Blood counts were done and the platelets found to have fallen to 50,000 to 82,000. The hemoglobin was 80 to 90 per cent. Two transfusions of 150 cc. of blood were administered and about a week after readmission she was transferred to the Department of Surgery, where a small piece of spleen was excised for biopsy. Mi-

croscopic examination of this showed a diminution in the size of the follicles, a profusion of large mononuclear cells in the sinuses (where they were actively phagocytosing erythrocytes) and a slight fibrous increase. A provisional diagnosis of "non-lipoid histiocytosis" was made for the occasion, as the picture was unlike anything we had seen or read about up to that time. The complete microscopic description will follow.

Two weeks later splenectomy was performed in the usual manner and the spleen sent to the laboratory of surgical pathology, where the following description was made.

"The specimen consists of a spleen weighing 310 gm.; it is distended and its capsule is tense, its margins softly rounded and its surface everywhere smooth and shining, with a slight mottling of lighter areas on a dark reddish background. On section it is firm and a good deal of blood escapes. The section-surface shows pearly gray areas about 3 mm. in diameter against a brownish red background.

Microscopically one encounters a very novel and obscure picture (Fig. 1): the capsule and trabeculae are somewhat thicker than normal, there is an increase in the connective tissue in the follicles and radiating from them. Four stains are employed: Masson's hematoxylin-phloxin-saffron, his anilin blue and his light green trichrome, and a combination of Mallory's phosphotungstic acid hematoxylin with a silver impregnation. The size of the Malpighian follicles is decreased, the arterial apparatus seems to be in order and practically all the pathological features are found on the venous side of the circulation.

The venous sinuses and the pulp surrounding them are inundated with large, polyhedral cells with a dense, rather than vacuolated, cytoplasm. This is somewhat basophil in the Masson stains. Their nuclei may be multiple, lobulated and double, or irregular and of the gigantic type; most of them are single and they are always vesicular and correspond with the typical nucleus of the retothelial cell. Many of them contain from one to many phagocytosed erythrocytes and, occasionally, ingested polymorphonuclear leukocytes. The silver impregnation shows these cells to lie in intimate relationship to complexes of reticulin fibrils, which further strengthens the assumption that they are retothelial. There is some increase in the reticular fibers of the splenic framework, particularly in the vicinity of the splenic corpuscles. The large phagocytic cells are not at all similar to those of Gaucher's disease as they are smaller, more discrete, denser and lack the characteristic longitudinal striations of these, as well as

their epithelioid grouping in the sinuses. They also lack the multivacuolated or spongy appearance of the cells seen in lipoid histiocytosis.

Besides the cells just described, one finds a good many eosinophil leukocytes and plasma cells in the pulp and sinuses, which gives the picture a slight cast toward the inflammatory side. Occasionally there is a lining up of the mononuclear cells along the walls of the intermediate venous sinuses and collecting veins, with a consequent similarity to cuboidal epithelium, a phenomenon not infrequently seen in low grade inflammations of the spleen. One more fact remains to be described: the presence of giant forms of mononuclear phagocytes that may be multinuclear, or may possess one very large vesicular nucleus, or a darkly stained and lobulated one, somewhat like that of the Dorothy Reed cell of Hodgkins' granuloma. We therefore have: (*a*) fibrous increase, (*b*) eosinophil leukocytes, and (*c*) cells resembling Dorothy Reed giant cells. Although these three points are characteristic of Hodgkins' disease, one is disinclined to make this diagnosis in the face of the other quite atypical findings and turns, rather, to some sort of inflammatory stimulus for an explanation. In the absence of precedent for diagnosis it would perhaps be best for the present to adhere to the biopsy diagnosis of 'nonlipoid histiocytosis.'"

Postoperative Course: After the splenectomy the platelets rose to 90,000, 120,000 and later to 300,000. The red blood cell count was unchanged, the leukocytes rose to 4000 and 9000 with 55 per cent adult polymorphonuclear and 8 per cent immature. The bleeding and clotting time were, as before, 3 minutes each. A culture taken from the wound before it had closed entirely showed *Staphylococcus aureus hemolyticus*. The child's temperature rarely exceeded 37.6° C, excepting on the 4th day after operation when it was moderately elevated. The blood cholesterol determination was 2.65 gm. per liter. The patient was discharged about 5 weeks after the operation, free from petechiae, and her wound healed over after a suture had come away.

She was readmitted 3 months later to the pediatric service as she had been complaining of pain in the legs after a fall that occurred a week or two earlier; she had also hurt her arm in a fall and it remained painful. She now exhibited a recrudescence of the petechial eruption and the liver had become still larger. A transfusion a week after readmission was followed by an aggravation of the petechial eruption over the face and trunk. Shortly after this a kyphosis was noted, tentatively diagnosed as tuberculous, and she was placed on a Bradford frame. The hip and shoulder joints became tender. There was subfebrile temperature, 37.6° C throughout her second hospitalization until death. A transfusion on June 3rd did not relieve the condition and death occurred on June 8th. She had a pharyngitis on May 16th, shortly after readmission, cultures from which

showed *Staphylococcus aureus hemolyticus* and *Streptococcus hemolyticus*; the staphylococcus was also recovered from the blood. The icteric index and fragility test were normal throughout her stay in the hospital. Roentgenological examination now showed rarefaction of the lower pelvis and of the right femur near its head, with osteoporosis of the spine and reticulation of the vertebral architecture, together with increase in some of the intervertebral spaces.

Autopsy Report

An autopsy was performed 3 hours postmortem.

Inspection: A well developed and well nourished white female of 3 years, 93 cm. in length and weighing 15 kg. No rigor mortis is present, nor is the kyphosis noted during life apparent. There is no increased flexibility in the vertebral column. Slight edema is present practically universally. The skin is white, except for innumerable small, discrete petechiae on the trunk, especially prominent over the chest. These are very small and distinctly outlined. No cervical adenopathy is noted, although there is slight enlargement of the inguinal nodes. There is no discharge from any of the body openings. A white scar is seen over the left rectus muscle, 12.5 cm. in length.

Incision: The usual Y-shaped median incision is made, as well as one over the length of the right femur; the head is opened in the usual manner. The subcutaneous tissue is edematous throughout and the fatty panniculus scanty. On running the knife parallel to the skin over the anterior thoracic wall a small pocket filled with reddish material of the consistence of cornmeal is encountered. This seems to arise from the right lower edge of the sternum and the adjacent ribs and is 3 cm. in its long and transverse axes and 1.5 cm. in depth. It does not extend to the pleural cavity. Another pocket is found over the lower left ribs; at first, thick chocolate-colored liquid is evacuated, followed by about 5 cc. of thick creamy fluid, which comes from the interior of one rib and does not involve the pleura. Cultures are taken from this. The costochondral junctions are soft but not cystic. Transection of the sternum shows deep purplish marrow in the manubrium, but none is identified in the gladiolus or xiphoid process.

Peritoneal Cavity: This contains about 30 cc. of clear fluid; there are a few fine adhesions present, especially around the cecum and the gall-bladder, the operative field about the splenic bed also showing a few. Slightly opaque areas are noted throughout the otherwise smooth and translucent peritoneum. The appendix is in its usual

position. The liver border shows two protuberant lappets separated from each other by a relatively retracted groove; one of them lies 7 cm. below the right costal margin in the midclavicular line, the other 6.5 cm. below the left rim in the midclavicular line, while the groove lies 7 cm. below the xiphoid process. The diaphragm is at the fifth right interspace and the sixth left rib. The mesenteric lymph nodes are moderately enlarged and white, the suprapancreatic group being the largest and having a pink color.

Thorax: The thymus weighs 10 gm. The pericardial cavity contains 50 cc. of watery, blood-tinged fluid in which some clots are found; it is thought that these originate from petechiae in the epicardium. There are no pericardial adhesions. The heart and aorta are not remarkable. The pleural cavities contain about 20 cc. each of clear colorless fluid. Aside from moderate enlargement of the peribronchial lymph nodes the pulmonary apparatus is unremarkable.

Organs of the Neck: The cervical lymph nodes are not much enlarged, the thyroid gland and organs of the neck are not removed, palpation and superficial inspection indicating that they are not abnormal.

Abdominal Organs: The liver weighs 1130 gm. Its lower presenting margin has been described; its consistence is firm, its color a light reddish and its section surface shows a definite increase in the connective tissue, with the formation of large lobules which cause a slight bosselation of the peritoneal surface and are of a light yellow color. Fine fibrous strands separate the masses of liver tissue from one another. The gall-bladder and its ducts are unremarkable. The spleen was removed at operation and has been described elsewhere. The pancreas is quite firm, but not otherwise remarkable. The suprarenal glands and the kidneys and urinary tract are also of the usual appearance, excepting for indistinct marking and pallor of the kidneys. The alimentary tract shows no notable departure from the usual appearance.

Head: The brain is not abnormal and exploration of the internal ears and accessory sinuses is unattended by the finding of anything of note. The skull shows no areas of porosity, or other changes.

Skeleton: The sternum has been described. The lumbar vertebral bodies contain uniformly dark purplish marrow, and after fixation small white spots appear on the section surface. There are cysts in each of the intervertebral cartilages examined, which are apparently

simple, unlined absorption defects (Fig. 2). The right femur contains masses of dark red bone marrow, particularly prominent at its lower end. There are no changes in the cavity of the right knee joint, its surface being everywhere smooth and shining. The left ilium contains no recognizable marrow; its spongiosa is quite dry and leathery.

Microscopic Examination

Thymus: There is marked fatty involution of the organ with necrosis and degeneration of the Hassal's corpuscles, which are difficult to find; a parathyroid gland of normal appearance is included in the section.

Heart: Nothing of note is seen.

Lungs: These are free from any definite bronchopneumonic areas and, aside from rather numerous mononuclear cells in the alveoli and scattered areas of interstitial lymphoid infiltration, they are unremarkable. No fat is demonstrable in the alveolar phagocytes.

Liver: The most striking feature in the sections is the large amount of fat in the parenchyma of the organ, globules being generally distributed throughout the section and swelling the liver cells to capacity. This may be slightly more marked on the portal side of the lobules than on the central venous side, but the distinction is not marked. The tissue between the liver cords is edematous and the Kupffer cells numerous and large, but not as prominent as in the case of lipoid histiocytosis. There are none of the crowded, epithelioid mononuclear cells that one observes in Gaucher's disease. Some of the numerous mononuclear phagocytes in the sinuses show a tendency to phagocytose erythrocytes, sharing in the picture of erythrocytic destruction evidenced in the spleen and lymph nodes, but to a far lesser degree. Most of the portal areas are infiltrated by lymphocytes and there is a definite interlobular fibrosis, demonstrable by Mallory's connective tissue stain. Frozen sections stained with Sudan III and Nile blue sulphate confirm the presence of large quantities of neutral fat in the parenchyma, but fail to demonstrate any of this in the Kupffer cells. No lipoids are demonstrable.

Pancreas: There is rather marked fibrosis of the organ with rather large numbers of mononuclear phagocytes in the fibrous tissue.

Suprarenal Glands: Examination of sections from these is negative.

Kidneys: There are the usual changes noted in toxic nephrosis, with numerous fat droplets in the epithelium of the convoluted tubules, which constitutes a lipoid nephrosis.

Mesenteric Lymph Nodes: Those about the pancreas show the most notable lesions (Fig. 3). The sinuses between the secondary follicles are greatly dilated and filled with large numbers of large mononuclear cells, which are actively phagocytosing erythrocytes in enormous numbers and, to a lesser extent, engulfing lymphocytes and other leukocytes. The follicles are compressed into mere ribbons between the greatly dilated sinuses. One section shows cells that are indistinguishable from megakaryocytes. Frozen sections stained with Sudan III and Nile blue sulphate demonstrate a little neutral fat in the capsule of the nodes, but none whatsoever in the phagocytes, which are also free from lipins.

Bone and Marrow: The intervertebral discs exhibit a simple loss of central substance, with a ragged wall to the cysts quite free from any cellular lining or inflammatory reaction. The adjacent vertebrae show a few fine bony trabeculae separated by very large spaces lined with endothelium and loosely packed with erythrocytes. Relatively few phagocytes are found in these sections. In those from the femur, on the contrary, there is considerable phagocytosis of erythrocytes by large mononuclear cells, together with relatively slight erythroblastic and only moderate myeloblastic activity. A few small areas of degenerating polymorphonuclear leukocytes (rather like tiny abscesses) are noted. Plasma cells are encountered in moderate numbers. There is early fibrosis of the marrow and occasional small masses of tangled fibrin are seen here and there. The general picture is reproduced in Figure 4. The sections from the sternum show, in addition, myxomatous degeneration of the marrow. The process in the marrow of these various bones, then, represents chiefly degeneration with a slightly inflammatory appearance, rather than proliferative activity. Supravital stains of the marrow add nothing to the picture.

Skin: No petechial hemorrhages are caught in the sections, but the derma contains many large mononuclear cells and lymphocytes and is edematous.

Special Stains: Sections of the bone marrow, lymph nodes and liver give a positive iron reaction by Perl's method, but the cells show none of the delicate, light blue lines characteristic of Gaucher's dis-

ease. The reaction is in the form of coarse blue granules in the phagocytes and differs in no way from that seen in the neighborhood of hemorrhages, or in cases of hemosiderosis from various causes.

Laboratory Examinations

Bacterial Examinations: Cultures were taken postmortem from the heart's blood and pericardial fluid and showed *Staphylococcus aureus hemolyticus* and a *Streptococcus hemolyticus* in both; others from the pockets over the ribs showed *B. proteus*, while those from the sternum showed *Staphylococcus aureus hemolyticus*. The *B. proteus* was a recent infection, the other two already having been found ante mortem.

Animal Inoculation: Emulsion of the liver was injected into the ear vein of a rabbit and into the peritoneal cavity of a guinea pig, emulsions of bone marrow being similarly inoculated into two other animals. All four were killed 27 and 29 days after inoculation without anything abnormal presenting at autopsy.

Summary and Diagnoses

Autopsy Summary: The outstanding features are: (1) Notable increase in mononuclear phagocytes in the spleen and the lymph nodes of the peritoneal cavity and, to a lesser degree, in the bone marrow. (2) The absence of demonstrable fat or lipins in the phagocytes. (3) Simple cystic degeneration of the intervertebral discs.

Anatomical Diagnoses: Healed splenectomy wound, moderate hyperplasia and hyperemia of the bone marrow, cysts of the intervertebral discs, petechiae in the skin over the trunk and in the epicardium, pericardial effusion, and granular degeneration of the tubules of the kidneys.

Discussion of the Diagnoses

This case, then, presents the following features already assumed to be pathognomonic of reticuloendotheliosis or aleukemic reticulosis:

Clinical Features: Universal petechial eruption on the skin; secondary anemia; enlarged liver, spleen and lymph nodes; subfebrile temperature; bony changes as determined by X-ray, and epistaxis (Borissowa[6]).

Pathological Features: Universal petechial eruption on the skin, petechiae in the epicardium; destruction of Hassal's corpuscles in the thymus (Letterer,[7] Guizetti[1]); generalized enlargement of the lymph nodes in certain regions; enlargement and fatty infiltration of the liver; large spleen; sclerotic changes in the liver and spleen, to a lesser extent in the bone marrow; bony changes already mentioned in three cases in the literature (*loc. cit.*) and pathological changes in the marrow. The microscopic picture of the spleen, liver, lymph nodes and marrow accurately corresponds with that described in most of the cases reported. The small necrotic foci over the ribs were also mentioned in one case (Letterer[7]) where there was a reddish phlegmon over a rib. With these facts in our possession it seems justifiable to add to the above anatomical diagnoses the more specific one of "non-lipoid histiocytosis," substituting this for the rather inclusive terms (reticuloendotheliosis, reticulosis, and so on) hitherto used for the same condition. For the same reason, it appears that we are dealing with an entity in the way of a disease and that we are entitled to add this case to those already reported.

Discussion of the Literature

Authorities are apparently agreed that the first cases to be published were those of Borissowa,[6] who described three under the caption "Beiträge zur Kenntnis der Bantischen Krankheit und Splenomegalie." Her findings were in close accord with our present conception of reticuloendotheliosis and decidedly at variance with the picture of an early Banti's syndrome. She described the phagocytosis noted in our case with meticulous accuracy and it is a pity that our present methods of tissue examination were not at her disposal thirty years ago when she wrote her paper.

As we have stated, there are two schools of thought as to the nature of this disease: the inflammatory and the leukemic. Hittmair[8] would extend it to cover infectious mononucleosis at the one end and diffuse sarcomatosis of the retothelial type at the other. Some authors consider it to be a sort of leukemia in which there may be three phases: the aleukemic (as typified by our case), the subleukemic, and the leukemic as represented by Swirtschewskaja[9] in her report in which 95 per cent of atypical mononuclear cells were circulating in the blood. Richter[10] reported a case in which there was

a general picture closely resembling reticuloendotheliosis, together with a typical lymphoid leukemia. On the other hand, such authors as Letterer,[7] Schultz, Wermbter and Puhl,[3] Krahn,[11] Akiba,[12] Goldschmid and Isaac,[13] and others, believe it to be of an infectious nature, a response to some low grade stimulus of a bacterial nature. Wihman [14] is one of those who will not commit himself as to the probable etiology.

The presence of subfebrile temperature in many cases, the history of some sort of infection preceding the development of symptoms, the similarity of the reaction to that often seen locally in lymph nodes draining chronic foci of inflammation or operative sites,—all these lead one seriously to consider an infectious basis for the condition, however alluring may be the conception that one is dealing with a third type of leukemia. Against the latter is the fact that there is a definite lack of anaplasia in the type cells (such as is seen in the true leukemias or in retothelial sarcomatosis), little evidence of mitotic activity and the lack of truly proliferative foci and immature forms of the type cells in the blood. One cannot overlook the fact that there is a vague similarity in the pictures of the lymph nodes and spleen to those seen in myelogenous chloroma or the localized myeloid tumors of myelogenous leukemia, but such areas are not found outside of the lymphoid apparatus in this non-lipoid histiocytosis, whereas they are almost the rule in the parenchyma of various organs in chloroma. Cases of true, generalized retothelial sarcomatosis are sometimes encountered and these have a totally different appearance from that of the disease under discussion, as well as a far more lawless distribution. Roulet [15] makes this comparison clear in his article on retothelial sarcoma, reporting a case similar to ours for contrast. Lack of invasion of epithelial or other tissue foreign to the reticuloendothelial system in such cases is perhaps the most telling point in favor of inflammatory origin, and seems to mark it as a response to toxic stimuli rather than a form of neoplasia.

We have quoted a number of articles in the body of this paper; there are others that may be referred to if the reader desires to pursue the subject. Perhaps the most complete bibliography is given by Hittmair,[8] who has reported a number of cases and made an extensive study of the disease. Besides those already referred to there are interesting articles by Tschistowitsch and Bykowa,[16] Ugriumow,[17] Ewald,[18] Benecke,[19] and Ungar.[20] Benecke's article deals with a case

of retothelial sarcomatosis, while Ungar's showed both a retothelial sarcoma of the humerus (with metastases) and the histiocytic hyperplasia of the disease under discussion. Both of these papers are interesting in connection with Roulet's two publications.

It is not our intention to discuss this question from the clinical standpoint; we have given as much clinical history as we deemed necessary for clarity and will leave its elaboration to our colleagues in the department of pediatrics. Our aim is to bring the case to the notice of American pathologists in the hope that the disease may be more readily recognized in this country and, perhaps, satisfactorily explained and placed on a more solid footing.

Microscopic sections of the frontal area of the brain were examined in silver impregnations after this paper was submitted for publication. They show a surprising increase in the number of microglia cells in the area examined, as well as an increased number of rod cells. Compound granular corpuscles were not found. The picture offered a striking similarity to that of general paresis and one quite unusual in so young a subject.

This examination was carried out by Dr. Lewis D. Stevenson, neuropathologist to the college.

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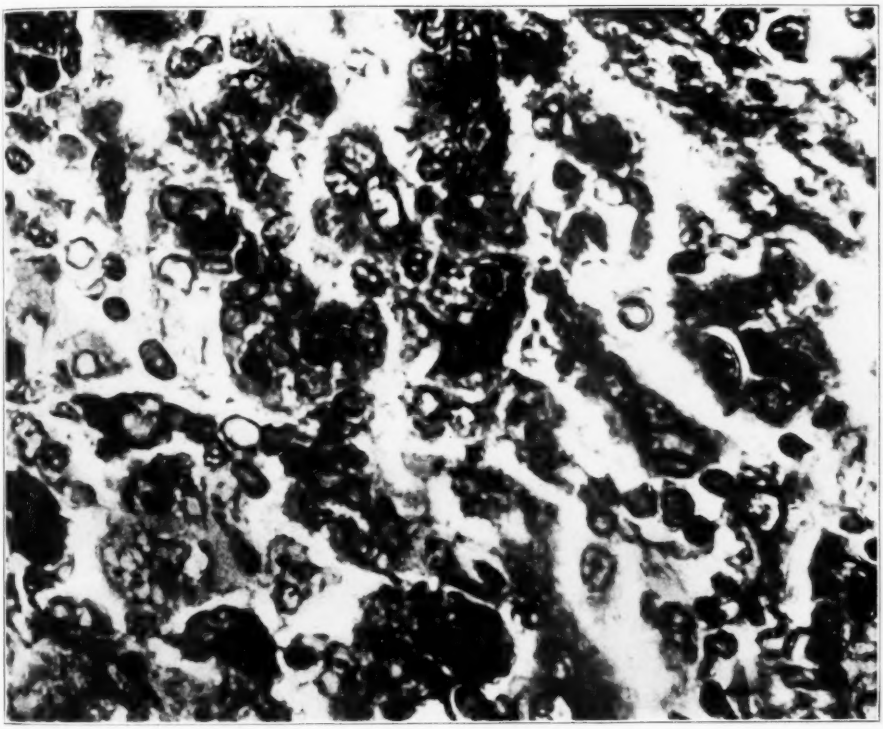
DESCRIPTION OF PLATES

PLATE 47

The photographs were made by Mr. William Dunn of the department of photography, Cornell University Medical College.

FIG. 1. Photomicrograph of the spleen. The erythrocytes are deeply stained and photograph black in most instances. Note the marked phagocytosis of these and the wiping out of the normal splenic topography by the masses of proliferated retothelial cells. Masson trichrome anilin blue. × 790.

FIG. 2. Photograph of the excised lumbar segment of vertebral column showing the cystic degeneration of the intervertebral discs and the whitish spots in the marrow of the vertebral bodies.



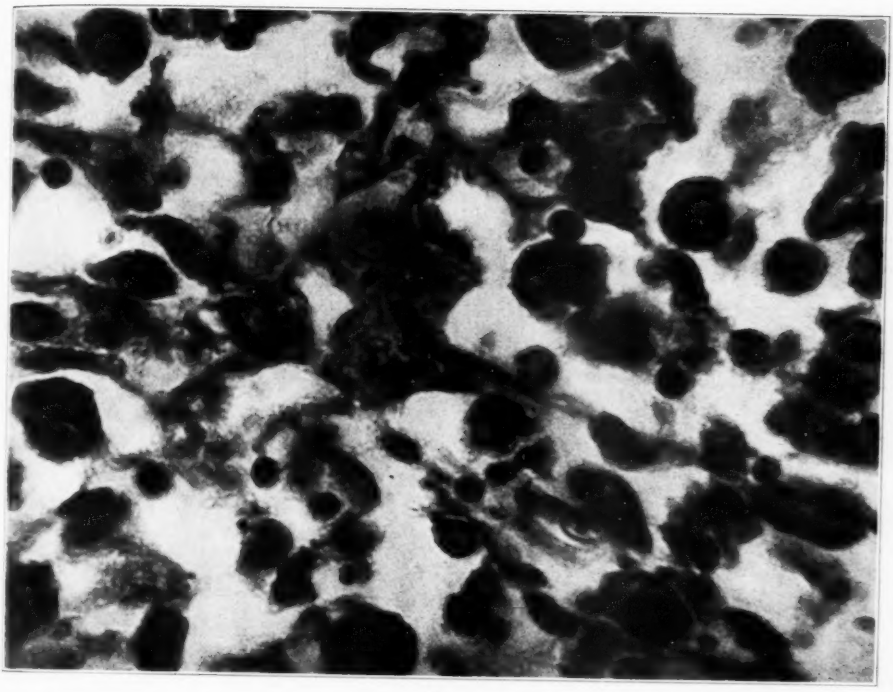
1

2

PLATE 48

FIG. 3. Photomicrograph of abdominal lymph node. The dilatation of the sinuses and the presence in them of phagocytes actively engulfing erythrocytes is very noticeable. Masson trichrome light green stain. × 350.

FIG. 4. Photomicrograph of the bone marrow of the femur. Phagocytosis is a feature here, although less marked than it is in the preceding figures. The topography of the marrow is much altered by the presence of numerous macrophages. Masson trichrome light green stain. × 790.



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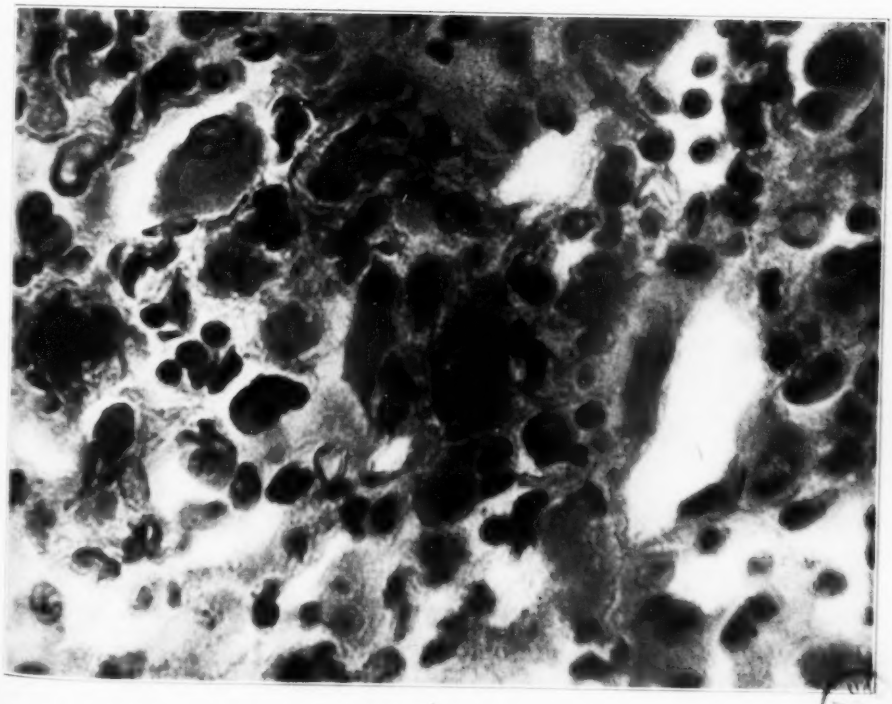

4

Foot and Olcott Non-Lipoid Histiocytosis

MULTIPLE BRANCHIOGENIC ACANTHOMA *

REPORT OF A CASE

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While acanthomas arising in branchial cysts are well recognized and not especially rare (we have seen 2 other cases, probably branchiogenic acanthoma, and 14 branchial cysts among some 2000 cysts and tumors of all kinds in this laboratory), it is believed that the occurrence of 3 such tumors in one patient in the space of 3 years merits reporting, especially as the tumors in question all arose definitely in cysts in different locations. Warren and Gates [1] list many cases of multiple acanthoma, among which are 4 in which 1 of the tumors was located in the neck. These were classed as cutaneous rather than branchiogenic, and there were no cases of multiple acanthoma of the neck, either cutaneous or branchiogenic.

The patient, a white seaman, aged 55 years, applied for treatment on April 28, 1930, for a small indurated ulcer of the lower lip. The ulcer was of about 3 weeks duration, and the indurated area was about 1 cm. in diameter. There was also a hard tender gland about 2 by 2.5 cm. in the left submaxillary region.

At operation at the United States Marine Hospital at Port Townsend, Washington, the lesion on the lower lip and a cyst about 2 by 2.5 cm. was removed from the left submaxillary region. The cyst ruptured in the course of removal. Its contents were sebaceous in character.

On Aug. 14, 1930, the patient returned to the United States Marine Hospital at Port Townsend, complaining of a lump of 5 weeks duration in the right submaxillary region. There was no evidence of recurrence of the lip lesion or of the tumor in the left submaxillary region. A second cyst was removed from the right submaxillary region, also about the same size as the first, and containing sebaceous material.

* Received for publication May 1, 1933.

After this the patient returned to sea and was not seen again until he applied for treatment at the United States Marine Hospital in Seattle, Washington, on March 1, 1933, complaining of a lump under his chin of 3 months duration. At operation a cyst about 3 cm. in diameter, containing necrotic material, not attached to the overlying skin, lying in the midline against the under surface of the symphysis of the mandible was found. A fragment of periosteum was removed with the cyst but the underlying bone was smooth.

The material received for histological examination consisted of the lip lesion and the three cysts mentioned above. Abstracts of the reports made are as follows:

"The lip shows only moderate downgrowth of the epithelium, with a few poorly limited prickle cell strands and an occasional pearl. The corium shows rather dense round cell infiltration and a few foreign body tubercles." The cyst from the left submaxillary region excised April 30, 1930, showed "an infiltrating tumor composed of cords and strands of prickle cells, with keratinized pearls and keratinizing small multilocular cysts. In one area this tumor is definitely invading a lymph gland."

The second cyst, removed on Aug. 15, 1930, from the right submaxillary region, presented "a cyst wall lined by a relatively well limited stratified squamous epithelium, and about this a malignant newgrowth composed of masses of epithelial cells with well defined prickles, cells with keratohyaline granules and keratinized pearls." Marked infiltrative tendencies and filling of lymphatic spaces were noted, mitoses were few, and well marked round cell infiltration of the stroma was noted. Adjacent scar tissue, fat, fasciae, muscle and hyperplastic lymph glands were not invaded.

The third cyst, removed piece-meal from the symphysis of the mandible on March 10, 1933, showed a multilocular structure. There were several small cysts filled with necrotic débris and lined or partly filled by foreign body granulation tissue and granulomas. There was a small lymph follicle in the wall of one of the cysts. The surrounding areolar, fascial and muscular tissues were fibrotic, infiltrated by lymphocytes and diffusely and irregularly invaded by strands and masses of poorly limited prickle cell epithelium with concentric keratinizing cell nests. Mitoses were moderately numerous. A small lymph gland and part of the excised muscle were not invaded.

The lip tumor was diagnosed as early epithelioma (Grade I); the

three tumors arising in cysts were all considered as definitely malignant and of about the same grade (Grade III). The first two appear to have arisen in cysts derived from the second branchial cleft; the last, lying in the submental region, probably represents a malignant transformation of a mesobranchial cyst. That these tumors represent independent primary growths is indicated by their grossly cystic nature and sebaceous contents.[2]

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BENZOL POISONING WITH HYPERPLASIA OF THE BONE MARROW *

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Chronic benzol poisoning is recognized as a potential cause of fatal leukopenic anemia, accompanied by aplasia of the bone marrow. Hamilton [1] in her recent review was able to collect only 35 more or less complete autopsy reports on cases of benzol poisoning. In 2 of these (Cabot,[2] and Martland, quoted by Hamilton,[1]) there was hyperplasia instead of aplasia of the bone marrow, although the blood picture did not differ in essential respects from the other cases. There has been some doubt as to whether or not the presence of bone marrow hyperplasia is compatible with the diagnosis of chronic benzol poisoning, and Hamilton left the question open for want of evidence. Thompson, Richter, and Edsall [3] have called attention to the frequency with which the blood picture of aplastic anemia is associated with marrow hyperplasia. A brief description of the following case is included in their paper, but the case was of such interest that it seemed advisable to report it in detail.

REPORT OF CASE

Clinical History: Presbyterian Hospital Case No. 364,427. E. B., an American-born, white male clerk, 53 years of age, entered the hospital on Dec. 29, 1932, with the complaint of weakness and bleeding from the nose and gums for 2 years. His family history was negative in regard to blood dyscrasias and was otherwise irrelevant. His past history was essentially negative, except for moderate nocturia for the previous 10 years, and for occasional nosebleeds since boyhood. These had lasted about 15 minutes and had occurred at intervals of several months. Later questioning revealed the fact that he was an amateur photographer and for 4 to 5 months prior to onset of illness had used benzol for cleaning bromoil brushes 3 or 4 nights a week in a closed room, without taking any precautions. Further inquiry revealed that the purchases of benzol began 5 years before entry to the hospital. The patient's family also stated that he had used benzol to remove the paint from the floors of his home. There was no exposure to it during the past year and one-half.

The patient's illness began 2 years before admission with a severe nosebleed lasting 18 hours, and finally controlled with adrenalin. His gums began bleeding at the same time. His physician kept him on a diet excluding red meat.

* Received for publication July 1, 1933.

TABLE I

Blood Counts

Date	Hemoglobin	Red blood cells	Reticulocytes	Normoblasts per 100 white blood cells	White blood cells	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Myeloblasts and myelocytes	Unclassified	Platelets
	per cent												
5/16/32...	48	2,650,000	..	3	1800	53	..	..	46	1	..	..	..
7/13/32...	..	....	..	..	..	..	..	..	..	..	..	..	65,000
7/9/32....	40	2,480,000	..	..	2000	41	1	..	56	2	..	..	..
7/23/32...	28	1,600,000	..	..	2800	37	..	2	49	12	..	..	..
8/1/32....	40	2,030,000	..	..	1000	28	..	..	68	4	..	..	30,000
8/8/32....	40	2,310,000	..	..	1800	32	..	..	64	4	..	..	14,000
8/15/32...	29	2,200,000	..	..	3100	19	..	..	79	2	..	..	14,000
8/24/32...	32	1,560,000	..	..	1500	48	..	..	50	1	1	..	..
9/10/32...	37	1,700,000	..	10	2200	59	0	..	39	1	1	..	6,400
12/29/32...	55	2,370,000	..	..	1800	52	..	..	44	2	2	..	18,000
12/31/32...	55	2,500,000	..	10	2200	28	1	..	66	5	..	..	..
1/9/33....	45	2,500,000	12.3	2	1700	42	..	..	56	2	..	..	18,000
1/16/33...	35	1,700,000	10.0	5	1400	66	..	..	30	4	..	..	21,000
1/18/33...	37	1,176,000	1.04	..	825	59	..	..	38	1	2	..	16,000
1/19/33...	35	1,170,000	4.1	1	1100	47	..	..	52	1	..	..	..
1/20/33...	32	1,392,000	0.2	5	1190	33	..	..	58	9	..	..	14,000
1/21/33...	..	....	..	..	3150	34	0.5	..	51	6	..	7.5	..

Until Aug. 24, 1932 the counts were done by Dr. E. J. Buxbaum of Jamaica, New York. The count of September 10 was done in the Shiel laboratory, Queens Village, New York. After admission on Dec. 29, 1932, the reports are from the Presbyterian Hospital laboratories.

One year later he had a carbuncle on the nose, which healed. In May, 1932, the first blood count was done and he was found to be anemic (see Table I). A course of liver extract and liver diet was prescribed at this time. During July, 1932, he received four transfusions without improvement in the blood picture (see Table I). He felt fairly well but gradually lost strength.

Physical Examination: Temperature 100.8° F, pulse 84, respirations 20, blood pressure 150/80. The patient was a well nourished man with a sallow complexion and dark brown pigmentation of the skin over the hands and ankles. The eyes were normal, except for mild sclerosis of the retinal vessels and a few patches resembling old hemorrhages in the fundi. The gums, teeth and tongue were somewhat coated and deeply stained. The heart and lungs were normal. The abdomen was distended with a palpable spleen and liver. The regional lymph nodes were not enlarged.

Laboratory Data: The blood counts are given in Table I. The red cells showed marked variations in size and shape with polychromatophilia and

TABLE II

Polymorphonuclear Counts (Method of Cooke and Ponder)

No. of lobes	I	II	III	IV	V	Total white blood cells
Normal	10	25	47	16	2	
Jan. 16, 1933	26	56	18	0	0	1400
Jan. 19, 1933	38	52	8	2	0	1100

basophilic stippling. Two normoblasts were seen. The polymorphonuclear cells were young. Polymorphonuclear counts were made on January 16th and 19th, after the method of Cooke and Ponder.[4] Fifty polymorphonuclear leukocytes were counted each time and a marked shift to the left was found, as is shown in Table II.

Bleeding time 2 hours, coagulation time 12.5 minutes, no clot retraction within 2 hours. Fragility test: hemolysis begins — patient 0.425, control 0.45; complete — patient 0.30, control 0.325. The blood Wassermann test was negative. Serum bilirubin: very faint trace. Blood chemistry: cholesterol 0.14 per cent, uric acid 3 gm. per 100 cc., serum protein 5.2 per cent, blood sugar 1.79 gm. per 1000 cc., serum calcium 11.1 mg. per 100 cc., serum phosphorus 3.3 mg. per 100 cc., blood non-protein nitrogen 22.1 mg. per 100 cc. Total plasma proteins 4.68 per cent, albumin 2.25 per cent, globulin 2.07 per cent, fibrinogen 0.36 per cent.

Urine: specific gravity 1.018, very faint trace of albumin on one occasion, otherwise negative. Stool: dark olive green, soft, guaiac 4 plus.

Course of Illness: The temperature was septic in type, ranging from 99.6° to 101.2° until the last week of life, when it rose irregularly to 104° F. The patient received the following treatment: A series of intramuscular injections of Lilly's liver extract, an intramuscular injection of 22 cc. of whole blood, three direct transfusions with a total of 1600 cc. of blood, and subcutaneous injections of anti-snake venom. Two days before death he was given 5 cc. of nucleotide intramuscularly. The gums continued to ooze and the nose to bleed to some extent. On January 17th he had a hemorrhage into the intestinal tract and

there was a bloody stool on the following morning. On January 18th he developed dyspnea and râles at the bases of both lungs. On January 19th he had a pain in the left side, which was thought to be a splenic infarct, and fresh petechial hemorrhages. A blood culture taken on January 20th showed *Streptococcus hemolyticus*. Death took place from respiratory failure Jan. 22, 1933.

Postmortem Examination

Autopsy No. 11,143, 12½ hours postmortem.

Gross Examination: The body is 175 cm. in length. The lips and nail beds are pale. Purpuric spots varying from pin-point size to 1 cm. in diameter are scattered over the body, but are more numerous over the thorax and abdomen and on the inner surfaces of the arms and thighs. They are not sharply outlined, are often irregular and vary in color. The feet, ankles, hands, and to some extent the face, are brownish in color without scaling or thickening. The sclerae have a faintly icteric tinge. The nose and ears are negative. The gums are purplish and spongy, with blood clots streaking over them, but without obvious ulcerations. The tongue is dry and black. The remainder of the external examination is essentially negative.

The abdominal subcutaneous fat is 5 cm. in thickness and contains many small hemorrhages. The peritoneal cavity contains 75 cc. of slightly cloudy, blood-tinged serous fluid. The right thoracic cavity contains 200 cc. and the left 300 cc. of a fluid similar to that in the peritoneal cavity, but they are not otherwise remarkable. There is a small mass of reddish, thickened connective tissue behind the aorta at the level of the diaphragm.

Heart: weight 540 gm. The enlargement is symmetrical and petechial hemorrhages are found in the epicardium, myocardium and endocardium. A very slight thickening and roughening in a small area of the anterior leaflet of the tricuspid valve is present. The valves are otherwise normal. There are a few sclerotic plaques in all the coronary vessels, which do not occlude the lumina.

Aorta: Normal.

Lungs: The right lung weighs 870 gm., the left 800 gm. Many small, subpleural hemorrhages are seen. The posterior two-thirds of all lobes of both lungs are firm, non-crepitant, mottled and moist. A small caseous nodule lies near the main bronchus of the right lower lobe.

Spleen: Weight 370 gm. Measurements 15 by 9.5 by 4.5 cm. The margins are rounded and the cut surface bulges above the capsule

and is moist. The pulp is soft but not friable, and contains many hemorrhagic areas. The malpighian corpuscles are distinguished with difficulty. No infarcts are found.

Liver: Weight 2580 gm. Measurements 28 by 19 by 10 cm. The organ is pale and the shape normal. It cuts with ease, is softer than usual, cut surface being moist and bulging above the capsule. The lobular pattern is distinct; the central areas are red, the portal areas form wider yellow zones. The bile passages are normal.

Pancreas: Normal.

Adrenals: Large, soft and friable. The cortex is gray with occasional small yellow nodules, and the lipoid content appears extremely small. There are many small, fresh hemorrhages in the adjacent fat.

Kidneys: The right kidney weighs 240 gm. and measures 13 by 7 by 3.5 cm. Both kidneys are normal, except for pallor and for many small hemorrhages in the pelvic fat and mucosa and a few in the kidney parenchyma. The left kidney weighs 240 gm. and resembles the right.

Pelvic Organs: The bladder is much dilated and hypertrophied and there are many small hemorrhages in the mucosa, the largest measuring 0.5 mm. in diameter. There is some prostatic hypertrophy. The testes and seminal vesicles are normal.

Gastro-Intestinal Tract: The stomach is much dilated and there are innumerable small hemorrhages in the mucosa, many of which have formed ulcers. These hemorrhages are also found in the first centimeter of the duodenum, but are not found in the remaining duodenum and small intestine. Similar hemorrhages are found in the cecum and to a less extent in the rest of the colon.

Neck Organs: Hemorrhages are present in the mucosa of the pharynx and the base of the tongue, and there is marked edema of the glottis. The *thyroid* is normal. The *lymph nodes* of the neck and axilla are somewhat enlarged, firm and bright red. The other lymph nodes are normal. Smears of the *sphenoid sinus* show great numbers of streptococci.

Bone Marrow: This was obtained from the sternum, the fifth rib, the lumbar vertebrae, and the femur at a point between the middle and lower thirds. It is similar in all these places, and is semifluid, pale red, and without fat. In the femur marrow there are a few hemorrhages.

Brain: Normal except for a few pial hemorrhages.

Microscopic Examination

The changes of greatest interest are found in the spleen, liver and bone marrow.

Spleen: There are many hemorrhages in the pulp. The malpighian corpuscles have tortuous and hyalinized central vessels and are edematous. Around the margins of the corpuscles there are many myeloid cells. Most of the abnormal cells have large, hyperchromatic, irregularly shaped nuclei surrounded by a little faintly basophilic cytoplasm. A few mature polymorphonuclears are found. The sinusoids are wide, with prominent endothelium, and they contain many clumps of nucleated red cells and megaloblasts. A few vessels are seen to be plugged with bacteria. Many of the cells in the pulp and a few in the corpuscles contain granules which stain for oxidase, and are especially well brought out by the dopa stain. The azure eosin stain reveals neutrophilic granules in most of the abnormal cells (Fig. 3). Phagocytes in the pulp contain much iron pigment.

Liver: The portions of the lobules around the efferent venules show extensive degeneration, often with complete loss of liver cells and with extensive hemorrhages. In these areas there are many polymorphonuclear leukocytes with multilobulated pyknotic nuclei. There are small numbers of myelocytes in the portal areas. The sinusoids contain many nucleated cells, some of which are normoblasts; others are myelocytes. A few sinusoids contain bacterial emboli. There is a little pigment in the Kupffer cells, which takes the iron stain. Most of the cells in the central half of each lobule and in the portal areas contain granules that take the oxidase stain (Fig. 4).

Lymph Nodes: The cervical lymph node contains many hemorrhages and many myelocytes. There are a few clusters of foreign body giant cells. The axillary lymph node is similar. The dopa and alphanaphthol pyronin peroxidase stains show that most of the myeloid cells are in the sinuses, though a few are in the pulp.

Bone Marrow: Marrow from the femur, rib, sternum and lumbar vertebrae is alike. All fatty marrow is replaced by blood cell formation. Many small islands of normoblasts are seen. Mature cells of the myeloid series are rare, but there are many nests of premyelocytes, cells with a large vesicular nucleus containing nucleoli and a pale pink, finely granular vacuolated cytoplasm (Fig. 3). Myeloblasts are few. Occasional small megalokaryocytes are found.

Many large phagocytes are stuffed with fragments of red cells and other débris. The azure eosin stain shows that the majority of the mononuclear cells contain neutrophilic granules (Fig. 4). Most of the cells give a positive reaction to the dopa stain and to the alphanaphthol pyronin peroxidase stain. Sections through the malleus incus and the setrous portion of the temporal bone show a similar marrow.

Other Organs: There is a recent bacterial vegetation on the tricuspid valve consisting of streptococci. Abscesses are found in the tonsil, epiglottis and behind the aorta at the level of the diaphragm. Bacterial emboli and hemorrhages are present in all the organs. There is an early bronchopneumonia, with very few polymorphonuclears in the exudate. The adrenals have undergone infectious degeneration. There are a few tubercles in the cervical and bronchial lymph nodes. The prostate is hypertrophied.

Anatomical Diagnoses: Aplastic anemia due to benzol poisoning; hyperplasia of bone marrow; hemorrhages in skin, subcutaneous tissue, heart, lungs, spleen, adrenals, kidneys, bladder and meninges; central necroses of liver; hemosiderosis of liver and spleen; acute sphenoiditis; acute tonsillitis; acute pharyngitis; abscess of epiglottis; acute bacterial endocarditis, tricuspid valve (*Streptococcus hemolyticus*); bacteremia (*Streptococcus hemolyticus*) with metastases; acute splenic tumor; infectional degeneration of adrenals; acute suppurative nephritis; erosions of stomach; bilateral hydrothorax; ascites; edema of meninges; lobular pneumonia; cardiac hypertrophy; benign hypertrophy of prostate; tuberculosis of cervical and bronchial lymph nodes.

Discussion

The essential features of this case are the same as in the 2 previously reported ones, namely, prolonged exposure to benzol, a leukopenic anemia, and active formation of both erythrocytes and leukocytes in the bone marrow, giving a picture suggestive of that in pernicious anemia. In Martland's case there were areas of both erythrocyte and leukocyte regeneration in the liver, and in Cabot's case there was erythropoiesis in the spleen and slight cirrhosis of the liver. In Cabot's case, which is reported in more detail, other points of similarity are present. The blood contained reticulocytes and normoblasts, in spite of the severe anemia with a hemoglobin of 35 to

50 per cent. The leukopenia of 2000–3000 white blood cells was due chiefly to a decrease of polymorphonuclear leukocytes. Platelets were much diminished and the bleeding and clotting time was prolonged. There was no clot retraction. Bleeding and spongy gums, petechiae in the mucous membranes and congestion of the liver, spleen and kidneys were present. It is perhaps worthy of notice that both of these patients were occupied in the production of artificial leather.

It is interesting to compare these 3 cases with the 2 reported cases of leukemia following exposure to benzol. The case of Delore and Borgomano [5] was that of a man of 41 years, who was exposed to benzol for 5 years in the process of preparing pyramidon. He developed hematemesis and was found to have multiple hemorrhages of the subcutaneous tissue, hematuria, and spongy and bleeding gums. The lymph nodes were enlarged. Examination of the blood revealed the following: hemoglobin 70 per cent, red blood cells 3,147,000, white blood cells 542,500, lymphoblasts 80 per cent, neutrophilic myelocytes 8 per cent, eosinophilic myelocytes 2 per cent, basophilic myelocytes 1 per cent, polymorphonuclears 6 per cent, lymphocytes 3 per cent. A few normoblasts were present. There was no clot retraction. Death followed gangrenous stomatitis. The autopsy revealed extensive hemorrhages in most of the organs, necrotic areas in the liver, and a large pale spleen. The bone marrow was not described. In view of the many myelocytes and few lymphocytes in the blood, and the difficulty of distinguishing lymphoblasts from myeloblasts, it is probable that this was a case of acute myeloid leukemia. It is impossible to be certain that the leukemia was the result of the exposure to benzol, but the circumstances are suggestive.

The second case, reported by Weil,[6] was that of a woman of 62 years who worked in the rubber industry. For about 2 years she had had fatigue, anemia, epigastric pain and loss of weight. On examination the essential findings were jaundice, a very large liver, and a spleen that extended below the umbilicus. The lymph nodes were not palpable. The red count was 1,080,000, hemoglobin 30 per cent, without anisocytosis, polychromatophilia, poikilocytosis, or nucleated red cells. The white count was 25,900, polymorphonuclears 60 per cent, lymphocytes 0.2 per cent, middle sized monocytes 32 per cent, large mononuclear cells 2 per cent, neutrophils 4 per cent.

After a series of transfusions and treatment with liver extract and X-rays, the red count rose to 1,240,000 hemoglobin, 40 per cent, and the white count dropped to 5600, polymorphonuclears and myelocytes to 81 per cent. The autopsy revealed a spleen and bone marrow with the lesions of myeloid leukemia. The bone marrow contained many large cells with a single pale nucleus containing nucleoli, and a scanty non-granular basophilic cytoplasm. Many megalokaryocytes, but few nucleated red cells were present. The splenic pulp contained masses of young myeloid cells and some megalokaryocytes. The liver was fatty. There was no evidence of myeloid cell accumulations in either the liver or spleen. There were no hemorrhages.

This case differs from the previous one in the absence of hemorrhages and in the greater maturity of the cells in the blood.

In view of Hamilton's [1] recent comprehensive review of the experimental work on benzol poisoning only a few of the most pertinent experiments will be mentioned. Selling [7] reported regeneration following aplasia in the marrow. Brandino [8] (quoted by Hamilton [1]) found complete regeneration of the cells in the marrows and spleens of dogs and rabbits after cessation of treatment with benzol. The recent attempts of Lignac [9] to produce leukemia in mice by means of benzol injections are of especial interest. After a number of experiments with various doses of benzol he injected a series of mice with minute amounts of benzol in olive oil once a week for periods of 4 to 10 months, and of the 54 mice used 8 developed some form of leukemia, and 2 more had large lymphoid nodules in various organs without change in the blood picture. An interval of 2 weeks to 2 months intervened between the last injection and death. Of the 8 leukemias, 3 were diagnosed lymphosarcomatosis; 1, lymphoblastic leukemia; 2, mast-cell leukemia; 2, aleukemic myelosis; and 1, eosinophilic myeloid leukemia. It seems discreet to reserve judgment as to the significance of these findings because of the frequent occurrence of various diseases of the lymphoid tissues in mice, and the fact that no mention was made of genetic control of the strain of mice. However, 3 or 4 of the 8 cases were types of leukemia that occur only rarely as spontaneous diseases in mice.

These experiments do not throw much light on the series of cases that have been discussed, except that they show that some degree of hyperplasia of the blood-forming tissues may occur after exposure to benzol. The work of Lignac suggests that hyperplasia may be the

result of repeated minute doses of benzol, but this is not true of any of the 5 cases discussed, for all were exposed to rather large amounts of benzol. It is conceivable that there may be some connection between the blood cell hyperplasia occasionally resulting from benzol and the occurrence of neoplasms as the result of the administration of coal tar. There is further evidence for this view in the experiments of Thomsen and Engelbreth-Holm,[10] who injected tar into the bone marrow of 62 chickens every 5 days for 2 to 3 months and found myeloid hyperplasia in 9 of them, and a blood picture resembling that found in spontaneous chicken myeloid leukemia in 1. This concept, however, must await further confirmation of the existence of leukemia resulting from benzol, since the occurrence of leukemia has not been proved to be more than coincidence, either in the 2 clinical cases or in Lignac's mice.

Summary

A case of benzol poisoning has been reported in which there was progressive diminution of the cellular elements of the blood, but in which the blood contained young cells and the bone marrow was extremely hyperplastic. Two other similar cases have been collected from the literature, together with 2 cases of leukemia associated with exposure to benzol.

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DESCRIPTION OF PLATE

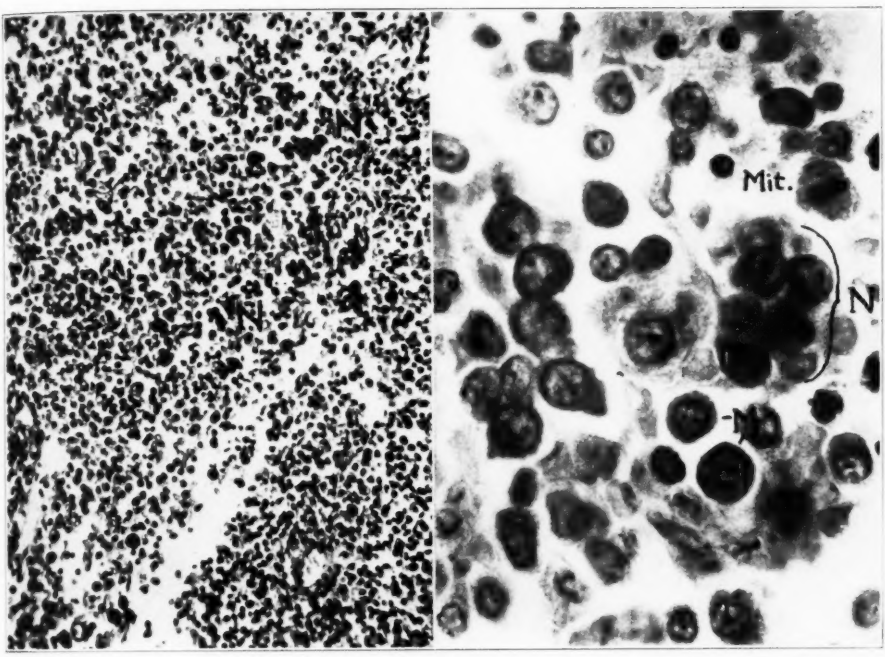
PLATE 49

FIG. 1. Bone marrow (femur). Among the masses of myeloblasts there are many nests of normoblasts (N). Azure eosin stain. × 160.

FIG. 2. Bone marrow (femur). This shows many myeloblasts (M), one of which is in mitosis (Mit) and one nest of normoblasts (N). Azure eosin stain. × 1050.

FIG. 3. Spleen, dopa reaction, showing the oxidase-containing cells around a malpighian corpuscle. × 160.

FIG. 4. Liver, dopa reaction. There are masses of myeloid cells in the portal spaces (P) and somewhat less concentrated groups of them in the central areas (C). A few cells in the sinuses also give a positive reaction. × 28.



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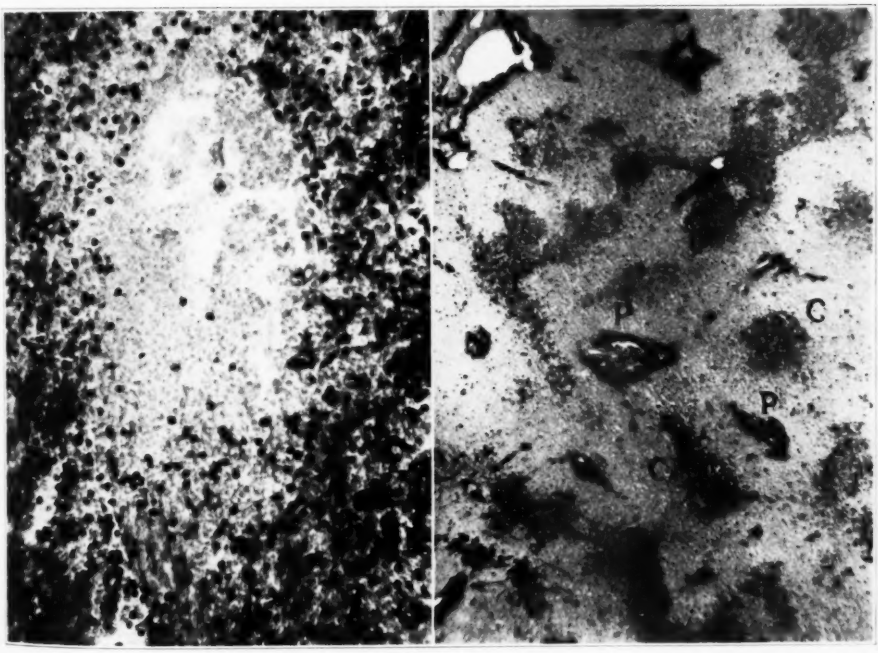

3 4

Andersen Benzol Poisoning with Hyperplasia of Bone Marrow

FRIEDREICH'S ATAXIA *

A CLINICAL AND PATHOLOGICAL STUDY

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The symptom-complex now known as Friedreich's ataxia was first described in 1861 as a slowly progressive weakness and ataxia developing during childhood and gradually advancing from below upward, attacking successively the legs, trunk and arms. Frequently more than one member of the same family is affected. It is further characterized by slow scanning speech, abolition of deep reflexes, scoliosis, foot deformities, integrity of the cutaneous sensations and absence of genito-urinary disturbances. Although many variations have been reported in the minor clinical features, the major symptoms are sufficiently constant to establish Friedreich's ataxia as a definite spinal affection.

The literature was reviewed by Barrett in 1927, from both clinical and pathological viewpoints. Since then numerous clinical histories have been published, but very few pathological studies are reported. In view of the long duration of the disease it is not surprising that pathological studies are infrequent. We have recently had the opportunity to study two cases of Friedreich's ataxia in sisters, one of whom died of an upper respiratory infection and came to autopsy. A detailed histological study of the nervous system was made.

CASE REPORTS

CASE 1. *Clinical History:* A. C., female, 21 years of age at the time of death, had been afflicted with this disease since the age of 5 years, and had been unable to walk since the age of 11 years.

Her early childhood presented nothing of particular significance. She was a full term baby delivered with forceps after a prolonged dry labor. However, there were no head injuries nor was there any respiratory difficulty. The birth weight was 9¼ pounds. She was breast fed for 14 months, gained weight steadily and developed normally. Her first teeth appeared at 4 months, she sat up alone at 5 months, talked at 10 months, and walked at 18 months. She had the usual infectious diseases of childhood — pertussis at 9 months, chickenpox at 2 years, measles at 5 years, mumps at 7 years, influenza at 8 years, and tonsillitis almost every winter. She was vaccinated at the age of 8.

* Received for publication August 3, 1933.

When 5 years old the first difficulty in walking was noticed — a certain weakness of the legs and slowness of the movements. Her ankles seemed to twist, her knees would weaken and give way and she would fall frequently; however, she never hurt herself in falling, was never unconscious and would immediately start walking again. The development and progress of the disease was so insidious that her mother did not become particularly worried about it until long after its appearance, when the weakness of the legs had become sufficiently developed to interfere seriously with walking. At this time the child was 7 years old and could hardly walk across the room without falling. She never complained of pain, which probably was the main reason why so little attention was paid to her condition at its onset. Coincidental with the onset of this ailment she developed a marked ichthyosis of the lower extremities, which persisted throughout life and resisted all attempts at treatment. Also, her spine started to assume that peculiar exaggerated lateral curvature so characteristic in these patients.

Two years after the onset of illness, at the age of 7, she developed coarse twitching movements of the entire body, especially in the extremities, which would become exaggerated upon walking. A physician consulted concerning these singular movements made a diagnosis of chorea.

At about this same time her speech became slightly impaired. At times she could talk normally, but often her speech was drawling and difficult. Hearing and vision were not affected. Mentally the child seemed very bright. She attended school regularly and kept up with her class easily.

When the patient was 9 years old the first complete physical and neurological examination was performed, the details of which will be recorded later. At this time the mother had already noticed that the patient was retarded in her physical development. She had developed a marked atrophy of all the muscles below the knees and her legs were always cold and cyanotic. The weakness in the lower extremities increased and her gait became more and more difficult until, at the age of 11, six years after the onset of illness, she could no longer walk alone and was confined to a wheelchair. The disease had progressed so slowly that it was not until she was confined to a wheelchair that she discovered that the trunk muscles were too weak to support her body in the upright position without the aid of the upper limbs. Because of the progressive weakness in these trunk muscles and the increasing difficulty of supporting her body she became bedridden at the age of 14 years and was confined to her bed for the remaining 7 years of her life.

In September 1929, at the age of 18 years, she suddenly lost the use of her entire right upper extremity with the exception of the fingers. The left upper limb remained normal so that she was still able to feed herself and perform various tasks. However, even this extremity grew weaker, and by September 1932 she was unable to use any of her four limbs. This was her final condition on Dec. 22, 1932, when she succumbed to an attack of pneumonia.

Throughout her illness she was always happy and readily adjusted herself to her steadily progressing ailment. Mentally she remained bright to the very end. She read a great deal, her eyesight remaining fairly normal. Physically (exclusive of this neurological condition) she was well, although her appetite was poor and her development somewhat retarded. The menses were established at the age of 15 years and continued regularly until the time of death.

The father, aged 57 years, and the mother, aged 52 years, are both living and well. The parents do not know of any member of either branch of the family

who had an illness resembling in any respect the affliction of their daughters. The patient had one brother, aged 23 years, who is living and very robust. However, her sister, aged 18 years, has had a similar affliction since the age of 11 years. The history of the sister will be given later.

The most complete physical examination was performed in 1921 when the patient was 9 years old, four years after the onset of illness.

The examination revealed a fairly well developed and well nourished white female child. Her face had a peculiar expression because she kept her mouth open and the front teeth protruded over the lower lip. Ears, nose and throat were normal. There was a slight enlargement of the posterior cervical glands. The lungs were normal. The heart was slightly enlarged to the left, with a faint blowing systolic murmur at the apex. The abdomen was negative. The skin of the lower extremities was notably cooler than that of the rest of the body. The skin below the knees was thickened, dry, coarse and scaly. The spine was not tender but showed a marked scoliosis with a slight kyphosis. There was an extreme degree of hypotonia and muscular weakness, because of which the child sat and stood with the shoulders sagging and the head dropped forward.

The urine was normal. The red cell count was 4,630,000, hemoglobin 71 per cent, leukocytes 14,800 — polymorphonuclears 70 per cent, lymphocytes 30 per cent. The Wassermann and von Pirquet tests were negative. Creatinin was 0.6 mg., sugar 96 mg., and urea nitrogen 12.07 mg. per 100 cc. of blood.

Electrocardiographic examination revealed a right ventricular preponderance and diphasic second and third leads. Roentgen-ray examination of the skull showed no evidence of tumor or of increased intracranial pressure. Roentgen-ray examination of the heart and lungs was also negative.

Neurological Examination: The patient was well behaved and very coöperative in the examination. Her memory and intellect were normal and she manifested an active interest in the various findings. Her vision was unimpaired, pupils reacted to light and accommodation, eyegrounds were normal, and there was no nystagmus. At intervals her speech became slow and somewhat scanned, but no stammering or hesitancy was apparent. All voluntary movements could be executed but were manifestly ataxic. Position and muscle sense, as well as the superficial sensations, were absolutely normal in all their manifestations. The deep reflexes were not elicited — biceps, triceps, supinator, knee jerks and ankle jerks. The Babinski test was negative, but the Romberg test was markedly positive. The child had an ataxic gait, walking with the legs far apart, advancing slowly and with difficulty, dragging her feet at each step and tending to walk on the outer aspect of one sole and the medial aspect of the other. Her feet were small and deformed, and showed a pes cavus. A general hypotonia was very evident.

From the age of 9 years the patient had occasional examinations. The only change noted was a progressive advance of the motor paralysis.

CASE 2. *Clinical History:* The second case is that of L. C., 2 years younger than her sister A. C. The onset and progress of illness was very similar to that of her sister.

L. C. was born normally at full term and was in good health during infancy. She developed normally and walked at an early age. Quite intelligent, she attended school regularly and soon learned to read and write. At the age of 11 the first difficulty in walking appeared. (At this time A. C., her older sister, was 13 years old and had already completely lost the use of her lower limbs.)

This weakness in the lower extremities progressed slowly, her gait became more irregular and hesitating and she tired easily and often would have to sit down and rest. Nevertheless, she was able to walk alone easily. At the onset of her ailment her skin, like that of her sister, became coarse, scaly and thick. This ichthyosis was generalized, resisted all treatment and has persisted to the present time. She has never complained of pain.

The earliest defect in the movements of the hands developed about one year after the onset of illness, at the age of 12 years. This started as tremors or oscillations which showed themselves toward the end of a voluntary movement, being most marked when she attempted some coarse action such as reaching for an object and grasping it. In addition to this tremor of the hands, she soon developed involuntary jerky movements like those of chorea in the muscles of the extremities and neck, the head showing marked jerking movements when she attempted to speak.

At the age of 13 years she finished grade school work, well up in her class, but because of the progressive weakness in her lower limbs she was unable to continue her education. She continued to walk for two more years until the age of 15, but her gait was becoming more and more difficult and finally, in 1930, her legs had become so weak that she could no longer walk alone. However, she could move the legs well when lying on her back. At about this time the first difficulty of speech was noticed. Her speech would at times become jerky and somewhat scanned. Occasionally, in talking, there would be a sort of hesitancy, then a series of words would succeed one another rapidly in an explosive manner.

During the last two years she has failed to develop physically and has remained small in stature. There has developed a marked pes cavus and an exaggerated curvature of the spine. The strength and usefulness of her upper limbs has been retained to the present time. The menses were established at the age of 15 years and have been regular since. Vision has been normal, although at times she complains of a slight blurring and diplopia. The lower extremities feel cold and often turn blue. At no time has there been any sphincter disturbances, although recently the child has complained of some urinary frequency.

She was examined thoroughly for the first time in 1928, at the age of 14 years, three years after the onset of illness. At that time the muscular development of the face and upper part of the body was good. The lungs and heart were normal on physical examination. Blood pressure was 92/52. The abdominal examination was negative. The skin over the entire body was dry and scaly.

The urine was normal. The red blood count was 3,900,000, hemoglobin 74 per cent, white blood cells 3800 — polymorphonuclears 50 per cent, lymphocyte 48 per cent, monocytes 2 per cent. The Wassermann reaction was negative. The basal metabolic rate was +28.

Neurological Examination: There was no impairment of vision. The pupils were equal and regular, reacting to light and accommodation. The fundi showed no changes. Hearing was normal and speech was characterized by its monotony, with the exception of occasional explosive expressions. Swallowing was unimpaired. There was a marked ataxia of the upper extremities; the biceps, triceps, supinator, knee jerks, and Achilles' tendon reflexes were all absent; the joint sense in the hands and feet was, however, normal. There was a marked reduction in vibration sense over the lower extremities. The

abdominal and skin reflexes were all present and unimpaired, and the muscle tonus was markedly diminished over the entire body.

The last physical examination was performed by us in January, 1933. The findings will be given in more detail in order to make clear her present condition, and the progress of the disease since the previous examination five years ago.

The examination revealed a well nourished but imperfectly developed individual. As she sat in her wheelchair one noticed an occasional oscillation of the head from side to side, and purposeless jerking movements of the upper extremities. This was associated with a peculiar twitching and an exaggerated play of the facial muscles, especially while talking, which would spasmodically draw the lips upward into an empty grin. The skin over the entire body was coarse, thick and scaly. The mouth, teeth and tonsils were normal; no enlarged lymph nodes were palpable. The breasts were well developed.

The pulse rate was 75 and normal in volume. The lungs and heart were normal. The abdomen was negative. The genitalia appeared normal externally.

The sense of smell was unimpaired. Visual fields were normal, visual acuity good, ophthalmoscopic examination negative. There was a marked lateral nystagmus with the quick component in a direction opposite to the gaze. Her corneal reflexes were greatly reduced. Superficial sensation over the entire face was unaffected, and the motor fifth nerve also seemed normal. Her hearing appeared moderately reduced, but the test was somewhat unsatisfactory due to the lack of coöperation and the conditions under which the test was performed. Vestibular tests were not done. No difficulty in swallowing was apparent and both pulse and respiration were unaffected. She was able to protrude the tongue normally, with no impairment of its side to side motion.

There was a slight generalized reduction in the size of the extremities, which appeared quite small in comparison with the trunk. A moderate contracture was present in the lower limbs, the lower leg being flexed on the thigh. The great toes were raised up clawlike bilaterally, with an extension of the metacarpal-phalangeal joint and a flexion of the interphalangeal joints. The feet were small, deformed and somewhat shortened, with the sole of the foot hollowed, producing a typical pes cavus. There was an extreme flaccidity at the wrist and elbow joints, accompanied by a typical foot drop; however, the muscular strength of the arms and legs was well preserved.

None of the deep reflexes was elicited even with enforcement, but the abdominal reflexes were present and normal. She had a bilateral positive Babinski and no clonus. All voluntary movements of the arms and legs could be executed although manifestly ataxic; it was impossible for the patient to touch with the index finger the tip of her nose or her other index finger even with the eyes open. She could not walk, her legs apparently being unable to support the weight of her body, but when sitting or lying there was no impairment of the movements in the lower limbs.

The loss of position sense in both the upper and lower extremities was very striking since she could not distinguish, with her eyes closed, even gross changes in the direction of her fingers and toes. The integrity of deep tendon and muscle sense was not disturbed. No definite impairment in the superficial sensibility was elicted, although the results of this test were somewhat unsatisfactory because of the marked coarseness of the skin and the lack of coöperation on the part of the patient.

On examination of her back one noticed the marked scoliosis with the convexity to the right in the thoracic region, and to the left in the lumbar region.

Her speech was slow and somewhat scanned, but without hesitancy. There was no stammering, but often a hesitation before an answer, and then a series of words would succeed one another with an almost explosive rapidity.

Discussion

The clinical history of our two cases furnishes a typical picture of the symptomatology of Friedreich's disease, which may be recapitulated as follows: A slowly progressive ataxia and weakness attacking two sisters, having its onset at the ages of 5 and 11 years respectively, beginning in the legs and extending gradually to the trunk and arms, but associated with almost no pain. There was also a slow, drawling scanned speech, choreiform unsteadiness, nystagmus, scoliosis, peculiar deformity of the feet (pes cavus), and abolition of the deep reflexes. To these we can add the negative findings of absence of pain, integrity of cutaneous sensation, integrity of sight, absence of genito-urinary troubles — the sphincter control being normal — and no luetic antecedents in the family. To complete the discussion of the clinical picture of Friedreich's ataxia a brief reference should be made to the literature since 1926 and to a few of the less frequent findings, such as occurred in our cases. About 25 cases that can be classified as Friedreich's ataxia have been reported since 1926.

In summarizing these cases the frequency of the most common findings was as follows: ataxia 100 per cent, loss or diminished deep reflexes 96 per cent, foot deformity 76 per cent, nystagmus 60 per cent, speech defect 60 per cent, spine deformity 56 per cent, positive Babinski 48 per cent, impaired deep sensation 36 per cent, impaired superficial sensation 12 per cent, muscle atrophy 16 per cent, and mental disturbances 4 per cent. Only one reference could be found in the literature to an accompanying ichthyosis which was so marked in both of our cases. Muscular atrophy was noticed only in our first case and as a rule is infrequent in this condition. The significance and interpretation of this atrophy will be brought out presently in the discussion of the pathological findings.

Because of the infrequence of detailed pathological studies in Friedreich's ataxia a careful report of our autopsy findings should be of interest.

Pathological Report

The autopsy showed an imperfectly developed and poorly nourished white female 143 cm. long, and weighing about 70 pounds. There was a marked scoliosis with the convexity to the right in the thoracic region, and to the left in the lumbar region. Both feet

Table I

Summary of Findings in 26 Cases of Friedreich's Ataxia

Author	Ataxia	Deformity of foot	Spinal deformity	Loss of deep reflexes	Nystagmus	Babinski's reflex	Speech defect	Impaired superficial sensation	Impaired deep sensations	Mental disturbances	Muscular atrophy
Beer, G.	+	..	..	+	..	..	+	..	..	?	..
"	+	..	..	+	+	..	..	..	+	+	..
"	+	+	..	+	..	..	..	+	..	..	+
Babonneix, L., & Schekter, L.	+	..	..	+	+	..	..	+	+	..	..
"	+	..	+	+	..	+	..	..	..	..	..
Alpers, B. J., and Waggoner, R. W.	+	+	+	+	+	+	..	..	..	..	+
" "	+	+	+	+	..	..	+	..	..	..	+
" "	+	+	..	+	+	..	..	..	..	..	..
" "	+	+	..	+	..	..	..	..	..	..	..
Hiller, F.	+	+	+	+	+	+	+	..	+	..	..
"	+	+	+	+	+	+	+	..	+	..	..
"	+	+	+	+	+	+	+	..	..	..	..
"	+	+	+	+	+	+	+	..	..	..	..
Popow, N. A.	+	+	+	+	..	..	..	..	..	..	..
Rabinowitsch, V.	+	+	+	..	..	+	+	..	..	?	..
Euziere, J.	+	+	+	+	..	..	+	..	+	..	..
"	+	+	+	+	+	+	+	..	+	..	..
"	+	+	+	+	+	+	+	..	..	..	..
"	+	+	+	+	+	+	+	..	+	..	..
Orbán, A.	+	+	..	+	+	+	+	..	..	..	..
"	+	+	..	+	+	..	+	..	..	..	..
"	+	+	..	+	..	..	+	..	..	..	..
Salus, F.	+	..	..	+	..	..	+	+	..	..	..
Krebs, E., and Mollaret, P.	+	..	+	+	+	+	..	..	+	..	..
Pommé, B., *et al*	+	+	..	+	+	..	..	..	+	..	+

showed a pes cavus. Each extremity measured 73 cm. from the anterior superior spine to the external malleolus. The left calf was 22 cm. in circumference, the right 20 cm. The left middle thigh measured 29 cm., the right 27.5 cm.; the left middle arm 18.5 cm., the right 17.5 cm. The muscles of the abdomen were fairly well developed.

The peritoneal cavity was normal. The diaphragm was at the level of the fourth interspace on the right, and the fifth rib on the left. The pleural cavities and pericardial sac were normal. The heart, root of the aorta and coronary arteries were normal.

The right lung weighed 250 gm., and its lower and middle lobes contained patchy areas of bronchopneumonia. The left lung weighed 150 gm. and was normal both externally and on section. Neither lung contained carbon pigmentation, bearing strong evidence of the indoor life of the patient.

The spleen, liver, kidneys and pancreas were all normal.

The muscles generally appeared fairly normal, with the exception of those in the lower extremities, which were largely replaced by adipose tissue. Microscopic examination of the atrophic muscles revealed a widespread but patchy degeneration of muscle with replacement by adipose tissue. In the persistent muscle bundles there were scattered groups of atrophic fibers in which the sarcoplasm had partially or completely disappeared. The sarcolemma shrinks to fit the reduced sarcoplasm but the muscle nuclei persist.

A striking change was found in the small intramuscular nerves. Almost all of these nerves showed a marked proliferation of the Schwann cells with replacement of nerve fibers, giving the nerve a sclerotic appearance. The structure of the nerves will be described more fully in a subsequent paragraph.

The entire spinal cord and many of the peripheral nerves were removed for further study. On gross examination the entire spinal cord was unusually small, appearing about one-half the normal size. The dura mater and the pia arachnoid were normal.

Sections were taken from various levels of the cord and stained in the following ways: hematoxylin-eosin, Weigert's myelin sheath stain, iron hematoxylin-Van Gieson, Nissl's method, azocarmine and Bielschowsky's stain.

The cervical cord presented the most marked changes. With the Weigert stain almost complete demyelinization was noted in the columns of Goll and Burdach, the lateral and ventral corticospinal tracts (crossed and direct pyramidal), and in the dorsal spinocerebellar tract (Fig. 1). The gray matter also presented a few alterations. The anterior horn cells were diminished in number, although none of them showed chromatolysis. No cells were visible in the posterior horns, which appeared somewhat vacuolated and contained a

number of dilated blood vessels. With the hematoxylin-eosin and the iron-hematoxylin-Van Gieson stains the cervical cord showed a moderate gliosis in the middle zone of the posterior and lateral columns.

The anterior columns were intact. The anterior rootlets were essentially normal. The myelin sheaths of the posterior rootlets were completely degenerated, while their axis cylinders were, for the most part, swollen or entirely absent. There was a marked Schwannian proliferation replacing many of the degenerated rootlet fibers.

No signs of inflammation were apparent. There was no cellular infiltration into the cord or around the blood vessels. The blood vessels themselves appeared normal.

In the midthoracic cord the changes were the same as in the cervical, except that demyelinization was not so pronounced in the pyramidal tracts. The anterior horn cells appeared normal both in number and in structure. An occasional shrunken nerve cell without a visible nucleus was found in the posterior horn. The cells of Clark's column were diminished in number, only a few appearing in the section. They were shrunken and irregular, the Nissl bodies were clumped, the nuclei, when present, were eccentrically placed (Fig. 3). There was only a minimal amount of gliosis present in the posterior and lateral columns.

The posterior rootlets were more involved than in the higher cord levels. They showed a marked destruction of both the myelin sheath and the axis clyinder, with a proliferation of the Schwann cells. In spite of the almost complete destruction of many posterior rootlets, a few areas were found with intact fibers. These persistent fibers probably accounted for the fact that some of the patient's superficial and deep sensations were intact. The anterior rootlets were unaffected.

The arteries around the cord were normal.

In the lumbar cord the posterior columns and the dorsal spinocerebellar tracts were still completely degenerated (Fig. 2). The crossed pyramidal tract, due to its actual decrease in size, appeared less prominent at this level. There was no sclerosis present. The anterior horn cells and the anterior rootlets were unaffected. Only a few intact fibers were found in the posterior rootlets (Fig. 2).

Some striking changes were found microscopically in the peripheral nerves. Only those of the extremities were removed for study

and, since the alterations in all were essentially the same, a detailed description of the changes in the sciatic nerve alone will be given.

With the Weigert's stain there appeared a patchy degeneration throughout the proximal part of the nerve (Fig. 4). In the distal portion, besides a similar patchy demyelinization, many of the nerve fibers showed a marked uniform swelling of their myelin sheaths. The axis cylinders were, as a rule, well preserved but in some regions, especially in sections taken from the distal segments of the nerve, they were somewhat swollen and often entirely absent (Fig. 4).

Another conspicuous feature in all the sections studied was the overgrowth of Schwann cells (Fig. 4). This proliferation was irregular and the manner of its distribution varied, even within a single section. In some funiculi this cellular overgrowth was limited to single or multiple well defined areas (Fig. 4), while in others it occurred as a diffuse proliferation which only partly replaced the nerve elements. In some areas, however, this Schwann cell proliferation was so marked that entire nerve bundles were replaced by large groups of cells and dense masses of fibrous tissue.

In the pathological reports found in the literature and well reviewed by Barrett, the most common lesions were a diminution in the size of the spinal cord, sclerosis of the posterior columns and degeneration of Clark's cells. Often a degeneration of the dorsal spinocerebellar tract occurred and less frequently a partial involvement of the crossed pyramidal tracts and posterior rootlets. Only rarely was there any mention of changes in the peripheral nerves. This may be due either to the absence of lesions or to the fact that the nerves were not examined at postmortem. Since Barrett's publication there have apparently been no autopsy reports on cases of Friedreich's ataxia, although there have been numerous clinical reports.

Friedreich himself called this complex hereditary ataxia. However, since his time it has generally been stated that Friedreich's ataxia is a familial but not a hereditary disease, appearing in families, usually involving a few members, but in many instances affecting a single individual. Indeed, from our histories it would seem that this was true, for there was no suggestion of a hereditary tendency in our two cases. But Friedreich's ataxia has been detected in several generations of a family as reported by Orbán, and others. These cases, although few in number, at least suggest a tendency to direct in-

heritance in this condition. Alpers and Waggoner have probably truly stated the situation when they write that Friedreich's ataxia is a "hereditary disease which often seems to appear sporadically in families, involving either a single or many members in the group."

Summary

1. Two typical cases of Friedreich's ataxia are presented, appearing in two sisters and presenting almost identical symptomatology.

2. A detailed postmortem report is given of one of the cases, with special emphasis on the histological changes in the peripheral nerves.

3. A brief summary is given of the literature since 1926.

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DESCRIPTION OF PLATE

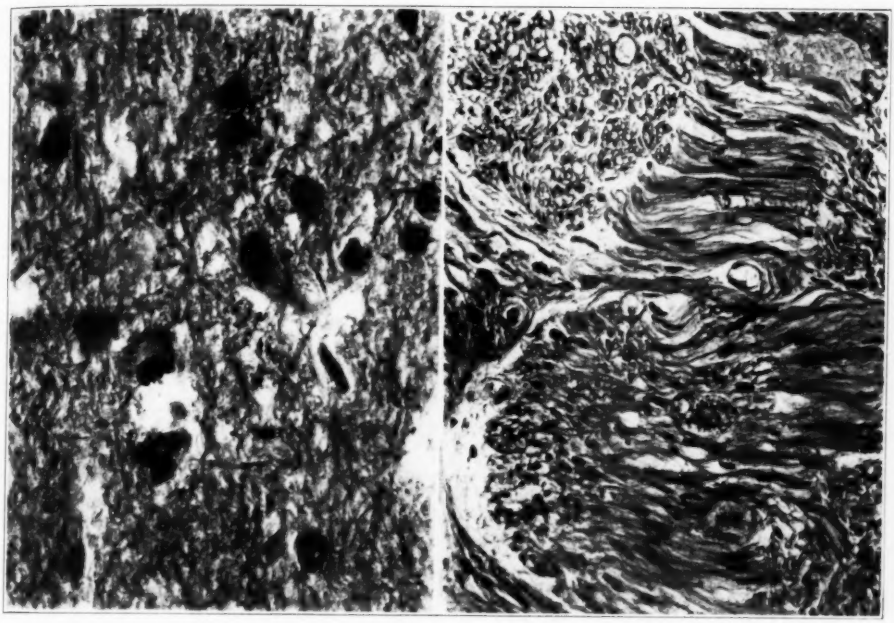
PLATE 50

FIG. 1. Section through the cervical cord. Weigert's myelin sheath stain. Note the destruction of the posterior and lateral columns.

FIG. 2. Section through the lumbar cord. Weigert's myelin sheath stain. There is a degeneration of the posterior columns and of the dorsal spinocerebellar tracts. Note the marked destruction of the posterior rootlets as compared to the anterior.

FIG. 3. Section through Clark's column in the midthoracic cord. There is a marked shrinking and irregularity of the cells as well as a reduction in their number.

FIG. 4. Section of the sciatic nerve. Iron hematoxylin-Van Gieson stain. Note the striking overgrowth of Schwann cells replacing the nerve fibers.



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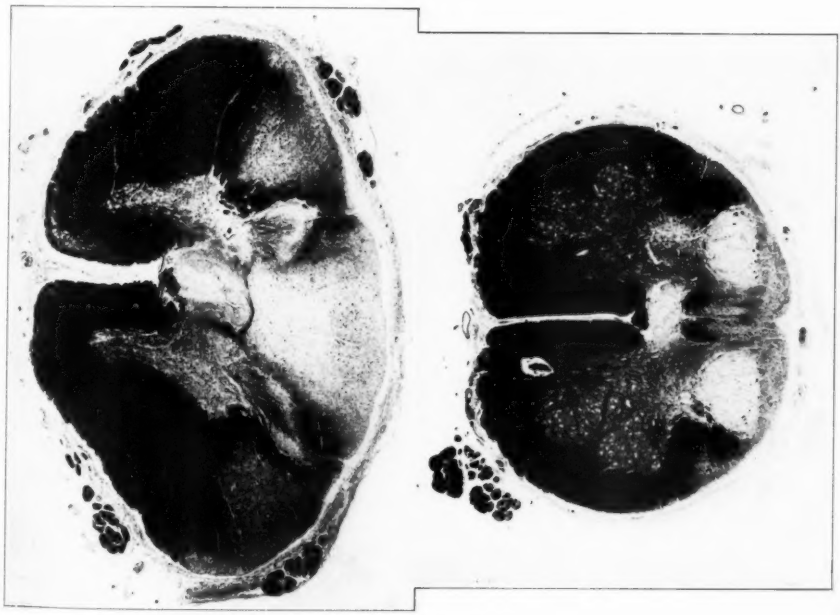

1 2

Baker Friedriech's Ataxia

PRIMARY NEUROGENIC SARCOMA OF THE BLADDER IN AN INFANT 1 MONTH OF AGE *

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The patient, a white female, 1 month of age, was admitted to the New Haven Hospital on July 7, 1932, gravely ill and in a condition of extreme malnutrition. The child was born in the hospital on June 7, 1932, after a protracted and difficult labor in which the mother's cervix was dilated by means of a Voorhees' bag and the delivery effected by breech extraction 24 hours after the onset of labor. The child cried spontaneously but because of cyanosis and partial pulmonary atelectasis carbon dioxide and oxygen were administered at intervals for 2 days. The physical examination at birth revealed a normal infant weighing 2810 gm. When feedings were started the child experienced some vomiting and diarrhea, but otherwise progressed favorably during the 10 days she remained in the hospital. Because of the mother's lack of intelligence little could be learned of the infant's condition prior to its return on July 7th. At this time the mother brought the child to the hospital after friends had called attention to its poorly nourished state. Physical examination revealed an extremely cachectic and acutely and gravely ill infant who weighed but 2330 gm. The temperature was unobtainable since it was below 34° C. The abdomen was scaphoid and soft. A firm, symmetrical, non-tender mass, which arose from the pelvis and extended halfway to the umbilicus in the midline was readily palpable. It was thought to be the distended bladder.

Immediately upon arrival at the hospital the child was given supportive treatment to combat the marked dehydration, but death occurred 18 hours after admission.

POSTMORTEM FINDINGS

The body was that of a well developed but poorly nourished female infant weighing 2350 gm. The skin over the body was thin, loose and

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inelastic but everywhere intact. There were no nodules, zones of pigmentation or nevi present in the skin.

The only significant changes in the viscera were confined to the genito-urinary tract (Fig. 1). When the peritoneal cavity was opened a firm, pale, ovoid mass, which extended 4 cm. above the symphysis pubis in the midline, was seen. This was the greatly enlarged and thickened urinary bladder, which measured 4 by 2 by 2 cm., and presented a uniformly smooth, even, external surface.

When the bladder was opened its wall was found to be thickened by dense masses of pale, translucent tissue that diffusely infiltrated the submucosa and extended haphazardly into the muscularis. The tumor masses appeared to be made up of pale yellow fibrils arranged in a whorl-like fashion. The wall of the bladder was irregularly thickened; the most extensively involved portions reached a diameter of 1.5 cm., while the less involved measured 0.5 cm. The mucosa of the bladder was everywhere intact and pale in appearance. In the thicker portions the tumor masses bulged into the lumen of the bladder and rendered its inner surface irregular in contour and greatly decreased its capacity. The greatest involvement was in the region of the trigone where the tumor masses impinged upon the ureteral orifices and produced a bilateral hydro-ureter and hydronephrosis. In this region the tumor had extended through the wall of the bladder to invade the vesicovaginal connective tissue and the outer layers of the cervical uterine wall. This extension did not alter the contour of the uterus or vagina, nor did it encroach upon the lumen of the urethra. The rectum was entirely free from invasion.

Microscopic Examination

The epithelium of the bladder is intact, but in many places it is raised in irregular fashion by masses of neoplastic tissue that infiltrate the submucosa extensively. The tumor tissue invades the muscularis and in several zones has reached the external surface of the bladder. The neoplasm is principally composed of bundles of fine pink-staining fibrils, throughout which are interspersed elongated fusiform cells with blue-stained oval nuclei. Clustered about these bundles are masses of cells with scant cytoplasm and large blue vesicular nuclei that contain a distinct chromatin network. These cells vary in size and shape. Many are round, others pyriform, and

frequently fibrillary processes appear to extend from them into the bundles. Occasionally small groups of these cells tend to simulate an acinar-like arrangement. Rarely cells are seen in a state of karyokinetic division. Distributed throughout the tumor are small numbers of large pyramidal cells with a dense homogeneous violet cytoplasm and large vesicular nuclei, in each of which a distinct central nucleolus is visible. Frequently these cells are multinucleated. In other portions the tumor is composed of smaller bundles of closely packed fusiform cells which present a wavy appearance. Usually associated with these are numerous ball-like structures made up of a central pink-stained fibrillar mass surrounded by one or more layers of spindle-shaped cells with pale blue oval nuclei. Frequently the fibrillary processes appear to extend from these cells into the central core.

In Mallory aniline blue preparations a distinct network of fine collagenous fibers permeates the tumor tissue and surrounds the various components which stain bright pink to violet.

Pal-Weigert myelin sheath stains demonstrate occasional myelinated nerve fibrils scattered throughout the neoplasm.

Discussion

The diagnosis of neurogenic sarcoma, in this case, is based on the presence of fibrillary structures associated with clusters of primitive cells and fragments of myelinated nerve fibrils which invade the wall of the bladder extensively. The masses of undifferentiated cells intermingled in bundles of fibrils, together with the large pyramidal cells and the ball-like structures, are features of the tumor that strikingly resemble the neoplasms described by Wright,[1] Pick,[2] Wolbach and Morse[3] and others, under the name of neurocytoma or neuroblastoma sympathicum. The presence of myelinated nerve fibrils, however, rather suggests the assumption that this tumor may not be derived from sympathetic nervous tissue elements. Furthermore, the scant amount of myelin present in the neoplasm may be due either to the embryonal nature of the tissue or to the fact that at the age of the patient myelin may not be formed. A source of origin of the neoplasm is readily accounted for in that the wall of the bladder has a rich plexus of myelinated nerve fibers derived from the lower sacral segments of the spinal cord. Although malignant tumors

arising from nervous tissue elements are known to develop in the adrenals and the retroperitoneal tissue of infants, the occurrence of such tumors primary in the wall of the urinary bladder has not been reported.

The rarity of primary neoplasms of the bladder in infancy is clearly brought out in Deming's review of the subject,[4] where only four instances are recorded in children under 1 year of age. Three of these were fibrosarcomas, the fourth a polyp. The only types of bladder neoplasms of nervous tissue origin so far reported are neurofibromas[5] associated with generalized von Recklinghausen's disease.

Summary

A case of neurogenic sarcoma arising in the wall of the bladder of an infant 1 month old is reported. This tumor resembles the group of neoplasms classed as neurocytomas, or neuroblastoma sympathicum.

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DESCRIPTION OF PLATES

Plate 51

Fig. 1. Bladder opened anteriorly to show main tumor mass bulging into trigone, with thickening of wall and associated bilateral hydro-ureter.

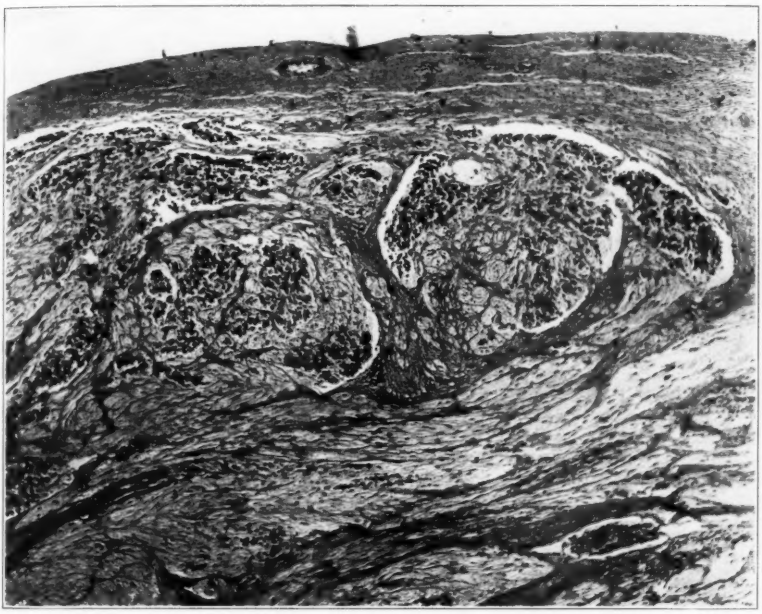
1

Harvey and Tennant Primary Neurogenic Sarcoma of Bladder

PLATE 52

FIG. 2. Tumor in submucosa of bladder wall showing bundles of fibrils and clusters of small undifferentiated cells. Hematoxylin and eosin stain. × 80.

FIG. 3. Large pyramidal cells and masses of small undifferentiated cells intermingled with fibrils. Hematoxylin and eosin stain. × 225.



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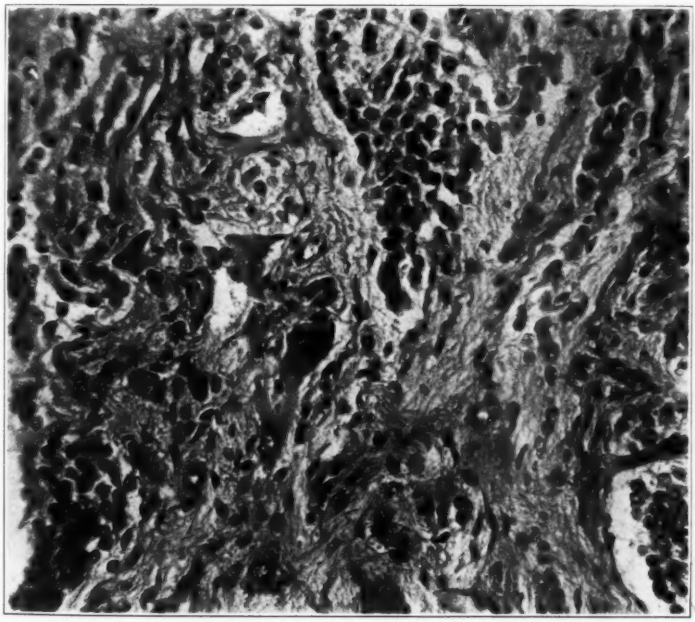


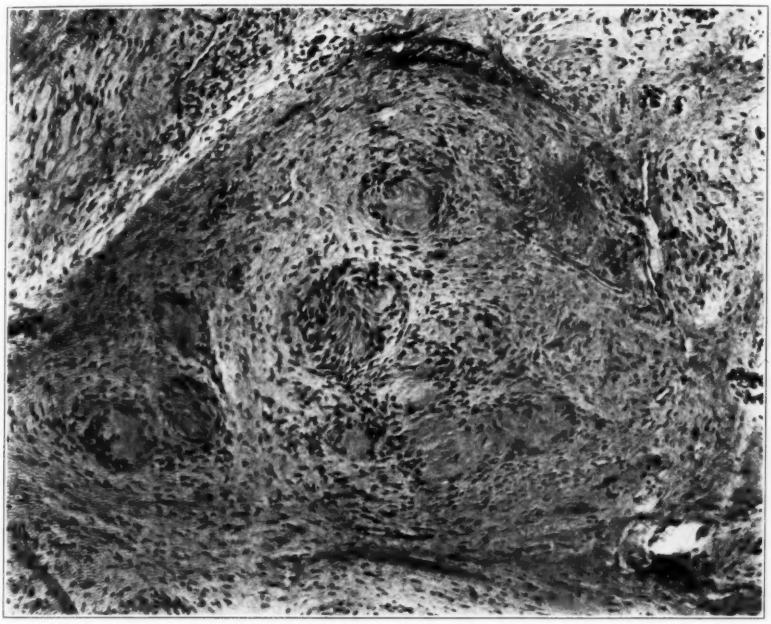
3

Harvey and Tennant Primary Neurogenic Sarcoma of Bladder

PLATE 53

FIG. 4. Ball-like structures with surrounding fibrillar bundles. Hematoxylin and eosin stain. × 125.

FIG. 5. Myelinated nerve fibrils in tumor mass. Pal-Weigert myelin sheath stain. × 150.



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5

Harvey and Tennant Primary Neurogenic Sarcoma of Bladder

THROMBOSIS AND PULMONARY EMBOLISM *

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Observations made upon a comprehensive series of autopsy cases from the services of a general hospital impress one with the fact that pulmonary embolism is a much commoner occurrence than it is ordinarily considered to be. Usually regarded as a postoperative complication of relatively rare occurrence it proves, on the contrary, to be a common autopsy finding, more often associated with medical than with surgical cases. The frequency with which it may be uncovered at autopsy is surprisingly high, as many pathologists have already pointed out (Lubarsch,[1] Fahr,[2] Henderson,[3] Singer,[4] Wertheimer,[5] Dietrich,[6] Benda,[7] Ceelen,[8] Axhausen,[9] Putnoky and Farkas [10]). On our own service, under the direction of Prof. Oskar Klotz, we have demonstrated pulmonary emboli in about 10 per cent of our routine autopsies and our observations have prompted this communication in the hope of drawing further attention to the seriousness of the problem, and in order to indicate some factors that seem of etiological significance.

The term pulmonary embolus is used here in a restricted sense, referring only to a wandering ante mortem blood clot of a size appreciable to the naked eye, lodged within the pulmonary arterial system or the right heart. It is impossible, as Dietrich [6] observes, to make a sharp distinction between infected and non-infected emboli. One can, however, make a rough but serviceable distinction on other than bacteriological examination between bland and septic emboli, and it is our purpose here to consider only those cases in which the emboli seemed of a bland character and did not arise from suppurative foci.

The frequency with which pulmonary emboli are found at autopsy depends to a great extent upon the vigilance and technique of the observer. There is, perhaps, no other autopsy finding which is more readily passed over. Unless the personnel of an autopsy service has

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TABLE I

Autopsy Cases of Chronic, Congestive Heart Failure with Pulmonary Embolism (Group 1)

Autopsy number	Age	Sex	Type of heart disease	Emboli of size to cause death	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung
	yrs.						
298/31	48	M	Hypertensive	+	Prostatic, leg veins and right heart		+
346	51	F	Rheumatic	+	Brachial and pampiniform	Swelling of arm	+
369	18	F	Rheumatic	+	Leg veins and right heart		+
371	45	M	Rheumatic	+	Leg veins	Varicose veins	+
414	70	M	Coronary	+	Prostatic and iliac veins		+
12/32	70	F	Coronary		Right heart		+
13	63	M	Coronary	+	Leg veins	Varicose veins	+
45	41	M	Rheumatic		Not found		+
66	36	F	Rheumatic	+	Leg veins and right heart	Edema of leg	+
107	46	F	Syphilitic aortitis	+	Right heart		+
143	71	M	Coronary	+	Right heart		+
241	51	M	Pericarditis	+	Right heart		+
254	23	M	Rheumatic	+	Not found		+
316	28	M	Rheumatic		Right heart		+
342	64	M	Coronary		Not found		+
378	60	M	Coronary		Prostatic veins		+
400	63	M	Coronary	+	Leg veins		+
21/33	43	F	Hypertensive	+	Femoral and ovarian veins		+
35	59	M	Coronary		Leg veins		+
36	68	M	Coronary		Jugular		−
48	60	M	Coronary	+	Not found		+
111	77	F	Rheumatic		Leg veins	Edema of leg	+
121	69	F	Coronary	+	Common iliac		+
131	54	F	Rheumatic	+	Leg and iliac		
139	68	M	Coronary		Prostatic		+

TABLE II

Autopsy Cases of Pulmonary Embolism in Cachectic or Debilitated Individuals with Various Fatal Diseases (Group 2)

Autopsy number	Age	Sex	Medical or surgical	Disease	Emboli of size to cause death	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung	Evidence of heart disease
	yrs.								
329/31	56	F	M	Liver cirrhosis	–	Not found		–	Acute verrucose, chronic sclerotic mitral endocarditis
368	24	M	S	Amyloidosis	–	Iliac vein		+	
48/32	90	M	S	Pyelonephritis with uremia	–	Iliac vein		–	Dilatation of right heart
130	59	F	M	Pyelonephritis with uremia	–	Femoral	Edema of leg	–	Dilatation of heart
158	69	M	S	Carcinoma of stomach	–	Leg veins		–	Brown atrophy of heart
191	49	M	M	Sarcomatosis	+	Femoral		–	Dilatation of right heart
291	49	M	M	Carcinoma of lung	+	Superior vena cava, pudendal		–	
338	53	M	M	Carcinoma of lung	+	Prostatic		–	Hypertrophy and dilatation of right ventricle
412	68	M	S	Carcinoma of stomach	–	Leg, pelvic		+	Brown atrophy and fibrosis of heart, passive congestion of liver
424	79	M	M	Brain tumor	–	Femoral	Edema of leg	–	Fatty infiltration of heart
16/33	68	F	M	Undetermined	+	Leg veins		–	Fatty infiltration of heart
19	75	M	S	Injury	–	Prostatic		–	Fibrosis of heart, diffuse dilatation of aorta
49	49	F	M	Diabetic gangrene	–	Common iliac	Gangrene of leg	+	Rheumatic heart disease
91	41	M	M	Sarcomatosis	+	Inferior vena cava		–	(Pressure on inferior vena cava)
124	40	F	M	Carcinomatosis	–	Iliac and inferior vena cava		–	Pericarditis
132	60	F	M	Sarcoma	+	Leg veins		–	(Pressure on inferior vena cava)

its attention especially directed to the problem there are bound to be many instances of pulmonary embolism that are not detected. Statistical studies of the autopsy incidence of pulmonary embolism, such as that undertaken by Rosenthal,[11] are therefore open to criticism on the grounds that figures from some clinics are much more carefully compiled than others. In another article [12] we have described at some length the special care that must be taken in order not to miss thromboses and emboli at postmortem examinations. Much has been written in recent years purporting to show an increase in the incidence of pulmonary embolism since the World War (see the reviews of Ceelen,[8] Dietrich,[6] Rosenthal [11]), but that such an increase may not be due to more careful autopsy examinations is open to question. To distinguish between embolus and autochthonous thrombus in the pulmonary arteries often presents difficulties, as has been indicated elsewhere.[12] Doubtful cases are, however, excluded from our series.

Our observations were made upon a series of 567 complete autopsies on adults during an eighteen month period ending April, 1933. In the series were 56 cases of pulmonary embolism. Thirty-seven of these showed emboli of sufficient bulk to occlude two thirds or more of the pulmonary circulation, and were therefore regarded as the immediate cause of death (Dietrich [6]). In the other 19 cases the emboli were small and of a non-fatal character. Medical cases predominated over surgical in the ratio of 40 to 16. In all but one or two cases there were multiple emboli, illustrating a fact, which we have previously emphasized,[12] that pulmonary embolism is, as a rule, not a single but a recurrent event, with repeated migrations of blood clot over a period of hours or days, leading up to a fatal termination in the event of two-thirds or more of the pulmonary circulation becoming occluded. Ljungdahl [13] described pulmonary embolism as being sometimes of a chronic nature.

A striking feature was the infrequency with which venous thrombosis was recognizable clinically. Probably as high as 60 per cent of the emboli arose from clotted leg veins (see Tables I, II and III), vessels relatively easy to examine and easy enough of detection when they are painful, yet the majority came to autopsy without recognition of the primary thrombosis during life. Three presented clinical thrombophlebitis with edema of one leg, and tender, indurated veins. Two more had gangrene of the lower extremities (due to arterial

TABLE III

Cases of Sudden Death from Massive Pulmonary Embolism in Convalescent Individuals (Group 3)

Autopsy number	Age	Sex	Medical or surgical	Disease preceding embolism	Days of illness	Site of thrombosis	Clinical evidence of thrombosis	Infarcts of lung	Evidence of heart disease
	yrs.								
361/31	50	F	S	Varicose veins injected	21	Leg veins	Varicose veins	–	Fatty infiltration
363	70	M	S	Bladder lithotomy	5	Not found		+	Coronary disease
400	76	F	S	Fractured femur	21	Leg and iliac veins		+	Fibrillation, fatty infiltration
87/32	58	M	M	Pneumonia	22	Femoral		?	
95	59	F	M	Pernicious anemia (remission)		Femoral		–	Fatty degeneration
117	30	M	M	Pneumonia	16	Not found		–	
170	58	M	S	Appendectomy	Few	Inferior vena cava		+	?
176	67	M	S	Stomach resection	3	Not found		+	?
229	44	M	M	Infective arthritis	Several	Leg veins		–	
284	67	F	S	Fractured femur	10	Not found		–	Fibrillation
386	64	M	S	Appendectomy	7	Leg veins	Varicose veins	–	
408	62	F	S	Ovarectomy	5	Leg veins		–	
23/33	64	M	S	Prostatectomy	2	Leg veins	Varicose veins	+	Coronary disease
110	44	M	S	Gastric resection	12	Internal iliac		+	
145	65	F	S	Fractured femur	8	Leg and iliac veins		–	Fatty infiltration

occlusion) and presented extensive venous thrombosis. Five additional cases were known to have varicose veins of the legs, but in no case were the varices tender nor did they show signs of active thrombophlebitis.

Of the 5 cases presenting evidence of active thrombophlebitis of the legs only 1 suffered a fatal embolism; the other 4 had but small emboli. As a general rule, the greater the local reaction accompanying a venous thrombosis the less the likelihood of large emboli breaking off. If, in and around a vein, there is an inflammatory reaction of sufficient intensity to create local manifestations the thrombus is likely to be securely attached to the vessel wall and only small masses are capable of dislodgement.

In the majority the thrombus was of the so-called spontaneous variety, developing without local signs. Four times out of five the resultant emboli were of a fatal character. Often enough it was impossible to determine the site of the parent thrombus, sometimes because it was not permitted to make a free dissection of the veins, sometimes because the entire thrombus had evidently migrated from its point of origin leaving no trace. In 8 cases the emboli closely resembled casts of femoral or saphenous veins but these vessels were found to be empty and apparently unaltered. Sixteen cases of spontaneous thrombosis with embolism showed remnants of thrombus in veins of the thigh. Six times the remnant was lying free in the vessel, ten times it was adherent to the intima and extended peripheralwards in one or more small tributaries. Similar adherent remnants were found three times in the internal iliac vein. Microscopic examination of these adherent thrombi showed no significant inflammatory reaction in the vein wall. The clot was made up of a compact platelet coagulum and was blended with the intima by a bland hyaline substance.

For purposes of further analysis our 56 cases fall into three fairly well defined clinical groups. The first and largest is the "heart failure" group in which 25 cases are found (Table I). All of these individuals were in a state of chronic invalidism from congestive myocardial failure, actual or impending. Twelve had coronary disease, 9 rheumatic heart disease, 2 hypertensive heart failure, 1 syphilitic aortitis and 1 pericarditis. Their ages varied from 18 to 77 years, with an average of 53. In only 5 of this group was there clinical evidence of venous thrombosis. The sites of thrombus

formation, as determined at autopsy, were as follows: leg veins 8, leg veins and right heart 4, right heart alone 5, prostatic veins 2, brachial and jugular veins each 1, not determined 4. In 15 cases the emboli were of a fatal character. All of this group except 2 showed infarcts of the lungs. In many instances the infarcts were of varying ages, indicating repeated emboli.

The second group comprises 16 cases, all of which were cachectic or debilitated with some incurable disease (Table II). Nine had inoperable malignant tumors. In 6 the emboli were large enough to cause death. The sites of thrombosis were: leg veins 6, femoral alone 3, iliacs alone 2, inferior vena cava 1, pelvic veins (prostatic, internal iliac, ovarian) 3. Three had infarcts of the lungs, 11 more showed pathological changes in the heart, so that 14 may be regarded as cases with impaired cardiac function. Two additional cases in the group, A–91–33 and A–132–33, had the inferior vena cava compressed by an intra-abdominal tumor. The emboli in each case arose from thrombi formed below the point of compression.

The third group comprises cases of sudden death from massive pulmonary emboli occurring in convalescent individuals who otherwise had a normal expectancy of life. Major interest attaches to these cases because of their dramatic clinical aspect. Fifteen cases fell into this group (Table III), 11 of them surgical and 4 medical. Six of the surgical cases followed laparatomies, 3 fractured femurs, 1 injection of varicose veins and 1 transurethral prostatectomy. The medical cases were made up as follows: 2 convalescent from lobar pneumonia, 1 recovering from infective arthritis and 1 pernicious anemia case in a phase of remission. The age variation in this group was from 39 to 76, with an average of 59. Three cases were known to have varicose veins of the legs, but in the other 12 there was no clinical evidence of venous thrombosis. Five of the 15 cases were diagnosed as embolism clinically. At autopsy venous thromboses were found in the following sites: leg veins 6, femoral alone 2, inferior vena cava 1, internal iliac 1. In 5 cases no thromboses were found. Six had infarcts of the lungs, a finding of twofold significance indicative firstly of repeated emboli occurring hours or days before the fatal issue, and secondly, affording evidence of impaired myocardial function to be considered more fully presently. In an additional 4 cases there was both clinical and pathological evidence of a damaged myocardium, 2 showing fatty infiltration, 1 fatty degeneration and

1 coronary disease. Thus, of 15 cases of sudden death from massive pulmonary emboli, 10 presented evidence of an impaired heart action.

Thirty-two out of 56 cases of pulmonary embolism had hemorrhagic, bland infarcts of the lungs. As has been shown by Karsner and Ash,[14] occlusion of a pulmonary artery is not productive of infarct unless the venous return from the lungs is impeded. Such an impediment may be brought about by compression or thrombosis of the pulmonary veins or by stenosis or insufficiency of the mitral valve. In our cases we were able to exclude, with reasonable certainty, obstruction to the pulmonary veins and were therefore obliged to account for the infarction on the grounds of inadequacy of the mitral valve. In 8 cases there was definite mitral stenosis and macroscopic evidence of chronic passive congestion (brown induration), but in the other 24 there was no demonstrable lesion of the mitral valve, save, in some instances, a moderate dilatation of the valve ring. A mitral systolic murmur had been noted clinically in a number of these cases.

The high correlation between heart failure and pulmonary embolism led to a consideration of all the cases of congestive heart failure in the series of 567 autopsies. There were 83 such cases. As we have seen, 25 of these had pulmonary emboli. An additional 11 cases showed thrombi capable of giving rise to pulmonary emboli; 7 had thromboses of leg or pelvic veins, and 4 mural thrombus of right auricle. Thus, out of 83 cases of congestive heart failure 36 had thromboses on the venous side of the circulation. In 20 of these cases the thrombosis occurred in leg or pelvic veins, and was discovered only at autopsy; there were no local manifestations during life and the microscopic examination of representative examples showed nothing more than a few lymphocytes as evidence of inflammatory reaction in the wall of the veins. In all probability there was a higher incidence of venous thrombosis in this group of cases than could be uncovered at autopsy. It is impossible to examine all the veins in the course of a routine autopsy and consequently it is impossible to determine the incidence of venous thrombosis accurately.

To summarize, 25 of the 56 cases were invalided with myocardial insufficiency, and 24 more presented evidence of a disordered heart with either infarcts of the lungs, or pathological changes in the heart (Tables II and III), or both; one case had a history of auricular

fibrillation up to the time of death but showed no demonstrable lesion. Thus, 47, or 84 per cent, may be regarded as having had impairment of cardiac function. Of this observation we shall have more to say later.

The main problem arising in connection with pulmonary embolism is the etiology of spontaneous venous thrombosis. Though our knowledge in this direction leaves much to be desired there are, without going into controversial points, several well established factors known to favor the process. Virchow [15] emphasized the importance of slowing of the blood stream as a factor predisposing to *intra vitam* coagulation of blood. Cohnheim,[16] and Ribbert [17, 18] showed that injury to the lining of a blood vessel afforded a localizing influence favorable, if not essential, to the development of thrombus. Aschoff [19, 20] determined a further condition, namely eddying of the blood stream, as propitious for the laying down of thrombus. Hueck,[21] and Dawbarn, Earlam and Evans [22] showed that an increase in the blood platelets synchronizes postoperatively with the period during which thrombosis is to be feared. There is now general agreement that two or more of these factors must operate in conjunction to be effectual; acting singly they are unproductive of thrombus. Besides these points it is common knowledge that thrombosis occurs with greater frequency beyond middle life than before, that it is prone to take place in the wake of operations, childbirth and acute infections.

From the present study it seems warrantable to emphasize another general principle concerning the occurrence of thrombosis, a principle that has received only casual attention in the literature, namely, that so-called spontaneous venous thrombosis has a high incidence in cases of actual or impending congestive heart failure. Virchow drew attention to a type of thrombus that developed in cachectic individuals, due, he thought, to a sluggish circulation, and he called it marantic thrombus. Welch [23] published a paper, "Venous Thrombosis as a Complication of Cardiac Disease." He reported 4 cases of his own and collected 23 more from the literature. Nearly all presented thrombophlebitis of the upper extremities with definite clinical manifestations. He was inclined to regard it as a relatively rare complication of heart disease, but it must be remembered he was dealing only with cases in which the thromboses had attracted attention during life, whereas in the majority of our own cases the thrombi

were incidental autopsy findings. Axhausen [9] found 270 instances of venous thrombosis in 1472 autopsies on cases of heart disease. Rosenthal [24] reported 94 instances of thrombosis in 149 autopsy cases of "cardiac decompensation," but did not indicate the nature or location of the thromboses, whether in heart, arteries or veins. Oberndorfer [25] observed that out of 97 medical cases dying of pulmonary embolism 64 were ill with cardiovascular disease at the time of the fatal seizure. He also is indefinite as to the nature of the cardiovascular lesions and their relation to venous thrombosis. Bauer [26] says 95 out of 100 cases of postoperative thrombosis and embolism observed by him had hearts that were not normal. He believes an impairment of cardiac function to be an important factor in the etiology of venous thrombosis, but does not discuss the nature of the cardiac abnormalities or their relation to thrombosis. Ophüls and Dobson [27] report cardiovascular disease in 52 per cent of cases of thrombosis and embolism, but again details are lacking. Putnoky and Farkas [10] found approximately 90 per cent of 91 cases of pulmonary embolism had cardiovascular disease and a large number showed fatty degeneration of the myocardium.

Our own figures show 36 instances of venous thrombosis in 83 autopsy cases of congestive heart failure and 49 instances of impaired cardiac function in 56 autopsy cases of pulmonary embolism. The dangerous type of venous thrombosis is that which develops without local signs and without demonstrable inflammation in the wall of the vein, and this type was observed 20 out of 83 times in association with chronic passive congestion of viscera, or anasarca, or both, due to heart failure. The same type of thrombosis was found 25 times in the other 484 autopsies of the series. Of these latter 25 cases, which constitute the bulk of Groups 2 and 3, 18 had evidence of cardiac inadequacy (see Tables II and III), though this was not the main cause of death. Thus in 46 cases of spontaneous venous thrombosis (with fatal embolus in 27) 20 were cardiac invalids and 18 more showed anatomical or functional changes in the heart.

What is the relation of cardiac incompetence to the development of venous thrombosis? To our knowledge this relation has never been defined beyond the general assumption that the circulation is slowed up. But what definite evidence is there that the velocity of flow in the veins is decreased when the function of the heart is impaired? To this question the work of Blumgart and Weiss [28] provides a definite

answer. These authors have shown that the circulation time is appreciably prolonged in cases of cardiac insufficiency, that the velocity of flow from peripheral veins to the right heart is definitely slower than in normal individuals, and that the venous pressure is increased. The observations of Blumgart and Weiss make it possible to postulate a slackened venous blood flow as a factor present in most of our cases of pulmonary embolism.

That the leg veins are the commonest site of thrombosis points still further to stasis as an important factor in the development of thrombosis. As Aschoff has pointed out, the veins of the lower extremities are the first to suffer a retardation of flow when the general circulation lags, partly because of the long column of blood in these vessels, partly because they may be compressed by Poupart's ligament, or in the case of the left common iliac vein, by the iliac artery. Other factors that tend to produce venous stasis in the lower extremities are increased intra-abdominal tension and immobilization of abdomen and legs. Of all operative procedures, incision through the anterior abdominal wall is the commonest to be complicated by pulmonary embolism. There were 6 instances of this complication in our series. Following laparatomy conditions are especially favorable for the development of thrombus in the leg or pelvic veins, probably because the venous return from the lower extremities is slowed up as the result of inertia of the abdominal musculature. The flow of blood up the inferior vena cava is retarded through diminution of the massaging effect of normal respiratory movements within the abdomen. If, in addition, the heart be embarrassed (as it was in 4 of our 6 cases), the venous return is still further retarded and stasis, then, unquestionably plays a major rôle. Fracture of the femur, which is not infrequently associated with pulmonary embolism (McCartney [29]), also has the effect of rendering leg and pelvis inert, thus favoring venous stasis. There were 3 cases of fractured femur complicated by fatal embolism in our series and in all 3 heart disease was present. There were also 2 instances of compression of the inferior cava by intra-abdominal tumors (Table II) and both suffered fatal embolism from thromboses developing below the point of compression.

Several clinical investigators (Walters,[30] Miller, [31] Polak and Mazzola,[32] Boshamer,[33] Fonio,[34] and Wright [35]), already convinced of the danger of venous stasis in postoperative cases, describe prophylactic measures aimed at maintaining adequate venous return from the

extremities. The administration of thyroxin to stimulate the circulation, elevation and massaging of the legs, application of heat and the encouragement of active movement are some of the measures employed in an attempt to prevent postoperative pulmonary embolism. Walters [30] reports encouraging results of such treatment at the Mayo Clinic.

Of the 56 cases that form the basis of this report only 11 had undergone operative treatment. Platelet counts were not made, but the factor of "tissue injury and the absorption of breakdown products" (cited by Dawbarn, Earlam and Evans [21] as the probable excitant of the postoperative platelet rise) was not present in the majority of our cases.

The work of Hueck,[21] and Dawbarn, Earlam and Evans,[22] has served to focus much attention on a relation between increase of platelets and a tendency to thrombosis. It should be remembered, however, that age does not influence the postoperative platelet rise; it is just as marked in the child as in the adult, and yet the latter is much more prone to develop thrombosis. Dawbarn and his associates state: "If the two factors of stasis and a high platelet level be combined in a given patient it would seem that thrombosis may be expected. In elderly patients the factor of stasis is probably the more important." It should also be borne in mind that no one, with the exception of Brock,[36] seems to have made observations on the platelet reaction in actual cases of thrombosis. Brock states that he has seen thrombosis in cases where the platelet count showed only a moderate rise, whereas other patients with a much bigger rise showed no evidence of any thrombosis. Willinsky [37] studied the platelet reaction in a number of postoperative cases and in general confirmed the observations of Dawbarn, Earlam and Evans, but none of his cases had thrombosis.

A large number of workers have directed their attention to changes in the coagulability of the blood, believing an altered state of the blood to be the chief factor responsible for thrombosis. While not denying the possibility of this factor playing a rôle it seems to us the fact is too often overlooked that thrombosis is a process essentially different from *extra vitam* coagulation of blood. The two types of coagula are widely different in origin and structure, as has been shown by Zahn,[38] Eberth and Schimmelbusch,[39, 40] Welch,[41] Aschoff,[19, 20] Apitz,[42] and Dietrich.[6] Dawbarn, Earlam and Evans,[22]

while purporting to show a correlation between the platelet count and clotting time, observed that patients with very high or very low platelet counts often showed but slight variations from the normal clotting time. The parallel which they believe to have established is not a striking one and there remains room for doubt as to whether the rapidity of *extra vitam* clotting varies directly with the tendency to thrombosis, whatever be the influence of the platelets.

The observations of Lubarsch [1] and Aschoff [19, 20] have done much to disprove the contentions of Welch,[23, 41] Dietrich [6] and others, that infection plays the most important rôle in the determination of thrombosis. In the majority of our cases the presence of infection could not be established as a definite factor. Furthermore, we sought to exclude from the present study those cases in which thrombosis arose as the result of suppurative lesions in relation to veins. Microscopic examination of the thrombosed veins failed to show signs of an active inflammatory reaction, thus affording further evidence of the absence of infection.

Summary and Conclusions

Pulmonary embolism is to be observed in approximately 10 per cent of autopsies upon adult individuals and is much commoner in medical than surgical cases. More than half of these cases show infarcts of the lungs and in nearly all there are repeated embolisms occurring over a period of hours, days or weeks. Thromboses of leg and pelvic veins are the chief source of dangerous pulmonary emboli. Such thromboses commonly develop without clinical manifestations and show no evidence of active inflammation in the adjacent vein wall. The dangerous type of thrombosis has a high incidence in cases of cardiac insufficiency. From the work of Blumgart and Weiss it is evident that the flow of blood in the veins is slowed when the heart's function is impaired, and slowing of the blood stream is known to favor thrombosis. Other factors seeming of lesser significance in our series of cases, we regard circulatory embarrassment as of prime importance in the etiology of venous thrombosis and pulmonary embolism. A high percentage of postoperative fatalities from pulmonary embolism show evidence of minor degrees of cardiac incompetence.

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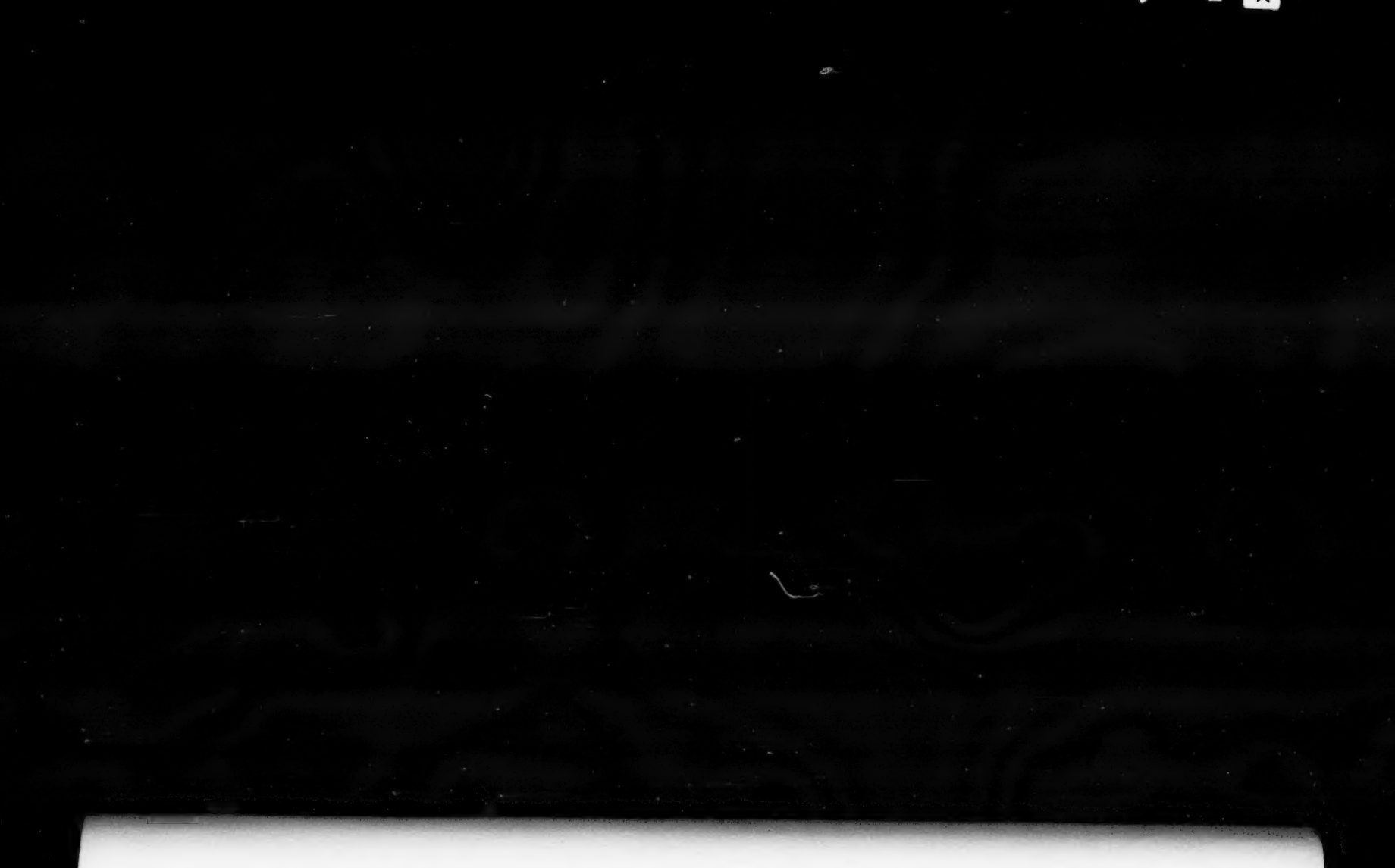
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